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(54) Title: CYCLIC COMPOUNDS AND COMPOSITIONS WHICH INHIBIT BONE RESORPTION

(57) Abstract

Described are new cyclic compounds that inhibit excess bone resorption and methods for the production of said compounds. Described are also compositions containing said compounds associated with pharmaceutical suitable carriers and methods for the treatment of diseases characterized by excess bone resorption, which method comprises administering to a subject in need of such treatment a bone resorption inhibitory effective amount of any of the novel cyclic compounds or a mixture of said compounds, optionally together with a pharmaceutical suitable carrier.

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CYCLIC COMPOUNDS AND COMPOSITIONS WHICH INHIBIT BONE RESORPTION.

This invention relates to new cyclic compounds that inhibit excess bone resorption and methods for the production of said compounds. Described are also compositions containing said compounds associated with pharmaceutical suitable carriers and methods for the treatment of diseases characterized by excess bone resorption and/or restricted calcium excretion, which method comprises administering to a subject in need of such treatment a bone resorption inhibitory effective amount of any of the novel cyclic compounds or a mixture of said compounds, optionally together with a pharmaceutical suitable carrier.

Typical diseases associated with the stimulation of bone resorption are osteoporosis, Paget disease of bones, humoral hypercalcemia of malignancy and metastatic bone disease. Increased bone resorption leads to brittle and weak bones which become susceptible to fracture and deformation. Hypercalcemia may lead to deposition of calcium within the kidneys and ultimately to impairment of kidney function.

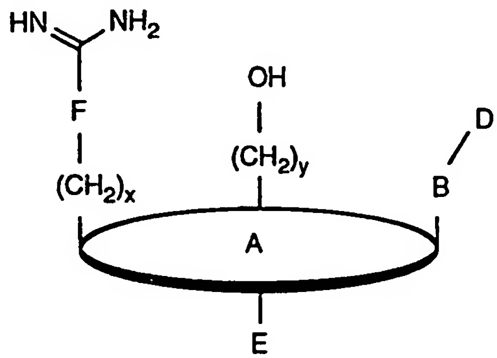
In principle diseases associated with the stimulation of bone resorption can be treated in 2 ways: either preventively by inhibiting bone resorption at a stage when bone is still sufficiently abundant with estrogens, bisphosphonates and calcitonins; or curatively, after substantial bone loss and fractures have occurred, by stimulating bone formation with fluoride.

The compounds mostly discussed at present for the treatment of such diseases are IGF (insulin-like growth factor), PTH (human parathyroid hormone) and PTHrP (human parathyroid hormone-related protein). PTHrP is a protein produced by a variety of cancers. It was first isolated and purified to homogeneity from a squamous carcinoma cell extract (Moseley et al., Proc. Natl. Acad. Sci. (1987), 85 5048-5052).

It was found recently (Fenton et al., Endocrinology (1991), 129 3424-3426) that the pentapeptide Thr-Arg-Ser-Ala-Trp (TRSAW), comprising the residues 107 to 111 of PTHrP is a strong inhibitor of bone resorption in rat. In WO 92/10511 several variants of

TRSAW have been described. The main disadvantage of the TRSAW fragments is their very low half life in rat sera due to proteolytic degradation.

Surprisingly, it has now been found that a cyclic compound of the formula (1)



(1)

wherein

A = cyclic radical having 12-17 ring forming atoms,

B = a spacer group, linked to the cyclic radical A via a carbon or nitrogen atom, having 1 to 6 carbon atoms and 0 to 2 nitrogen and 0 or 1 oxygen atoms in the backbone of the chain; wherein the carbon atoms can be substituted by oxo, hydroxy, sulfo, C_1-C_4alkyl, morpholino, amino, carboxy and/or by a radical of a naturally occurring amino acid or a radical of a peptide containing 2-5 naturally occurring amino acids; and the nitrogen atoms can be substituted by C_1-C_4alkyl, C_1-C_4alkanoyl, e.g. formyl and acetyl, and/or by a radical of a naturally occurring amino acid or a radical of a peptide containing 2-5 naturally occurring amino acids;

D = aryl-C_1-C_4alkyl wherein the aryl radical is mono- or bicyclic and unsubstituted or substituted with OH, SH, NH_2 or halogen, e.g. F, Cl, Br, I;

E = NH_2; NH_2 substituted with C_1-C_4alkyl or amino-C_1-C_4alkyl; C_1-C_4alkyl substituted with one or more, especially one or two, amino groups; a five- or six-membered one or two nitrogen containing heterocyclic radical; or NH_2 substituted by an acyl radical of a naturally occurring amino acid or an acyl radical of a peptide containing 2-5 naturally occurring amino acids;

F = NH or CH_2;

x = 1 to 6;

y = 0 to 4;

with the proviso that the residues bearing the $(CH_2)_x$-F-C(=NH)NH_2, $(CH_2)_y$-OH and B-D radicals are located above the plane assumed by the cyclic radical A and the

radical E is located below the plane assumed by the cyclic radical A;
or a pharmacologically acceptable salt thereof;
is distinguished by an excellent stability and an inhibition of excess bone resorption.

Cyclic radicals A embraced by the invention include for example crown ethers, steroids, porphyrines and cyclic peptides. In preferred cyclic radicals the distance between the binding sites of the B-D-group to the cyclic radical and the E-group to the cyclic radical is 0.50 to 0.65 nm, preferred 0.55 to 0.60 nm, the distance between the binding sites of the $(CH_2)_y$-group to the cyclic radical and the E-group to the cyclic radical is 0.50 to 0.62 nm, preferred 0.55 to 0.60 nm, and the distance between the binding sites of the $(CH_2)_x$-group to the cyclic radical and the B-D-group to the cyclic radical is 0.55 to 0.75 nm, preferred 0.60 to 0.70 nm.

Especially preferred are cyclic peptides (arranged head to tail) or peptides wherein the side chains of two amino acids or the side chain of one amino acid and the N- or C-terminus of another amino acid are connected directly or via a linking group. To avoid rapid degradation in the organism, e.g. by proteolytic enzymes, one or more, e.g. 1-5, genetically encoded amino acids can be replaced by non genetically encoded amino acids; or two or more, e.g. 2-3, amino acids can be replaced by a suitable ω-amino-carboxylic acid which is substituted to fulfill the steric requirements of formula (1). In a preferred embodiment of the invention the cyclic radical comprises a mixture of D- and L-forms of amino acids.

Linking groups are for example CH_2, C_2H_4, C_2H_2, $CH(CH_3)$-CH_2, O, CH_2-O, C_2H_4-O, CH_2-O-CH_2, S, CH_2-S, CH_2-S-S, S-S-CH_2, S-S, S=O, O=S=O, NH, NH-CO and Se.

Amino acids are for example all naturally occurring amino acids, e.g. genetically encoded amino acids like alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophane, tyrosine and valine; the D-forms of said amino acids or amino acids like 2-aminoadipinic acid, 3-aminoadipinic acid, beta-alanine, 2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisobutyric acid, 2-aminopimelic acid, 2,4-diaminobutyric acid, desmosine, 2,2'-diaminopimelic acid, 2,3'-diaminopropionic acid, N-ethylglycine, N-ethylasparagine, hydroxylysine, allo-hydroxylysine, 3-hydroxyproline, 4-hydroxyproline, isodesmosine, allo-isoleucine, N-methylglycine, N-methylisoleucine, 6-N-methyl-

lysine, N-methylvaline, norvaline, norleucine, ornithine, citrulline, γ-carboxyglutamic acid, o-phosphoserine, homoserine, homocysteine, carboxyethyl-piperidine, amino-iso-butyric acid, diamino-propionic acid or N-(3-glycinyl)propylguanidine. The conformation of the amino acids is chosen such that the steric requirements of the compound of the formula (1) are fulfilled.

In case spacer group is linked to the cyclic radical A via a carbon atom, the spacer group B can be a C_1-C_6alkyl radical, e.g. methylene, ethylene, propylene, butylene, pentylene or hexylene. The spacer group can comprise up to two nitrogen and/or one oxygen atom in the backbone of the chain, e.g. -CH_2-NH-, -CH_2-CH_2-NH-, -CH_2-NH-CH_2-.

In case spacer group is linked to the cyclic radical A via a nitrogen atom, the spacer group B can be a radical of the formula -N(H or C_1-C_4alkyl)-(C_1-C_4alkyl) radical, e.g. -NH-CH_2-, -N(CH_3)-CH_2-, -NH-CH_2-CH_2-, -N(CH_3)-CH_2-CH_2- and -NH-CH(CH_3)-CH_2-.

In a preferred embodiment of the invention the spacer group has 2 to 6 atoms in the backbone and in a more preferred embodiment the spacer group is the backbone of an amino acid chain. One or more carbon or nitrogen atoms of this spacer group can be substituted by C_1-C_4alkyl like methyl or ethyl; carboxy and/or a radical of a naturally occurring amino acid or a radical of a peptide containing 2-5 naturally occurring amino acids. The amino acid radicals and the peptide radicals are preferably bound via an amide linkage. The carbon atoms in the spacer group can be additionally substituted by oxo; hydroxy; sulfo and amino. A part of the first amino acid can also be a member of the heterocyclic radical A.

The aryl radical in D is, e.g. a five- or six-membered monocyclic radical or a bicyclic radical consisting of two five- or two six-membered rings or one five- and one six-membered ring. Examples for aryl radicals are phenyl, biphenyl, isobenzofuranyl, chromenyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolizinyl, isoindolyl, indolyl, indazolyl, purinyl, isoquinolyl, quinolyl, phthalazinyl and naphthyl. These aryl radicals are unsubstituted or substituted by C_1-C_4alkyl like methyl or ethyl, hydroxy, sulfo, amino and/or carboxy to give, e.g. the corresponding radicals of phenol, aniline, napthoquinone, phthalimide, phthalic acid, salicylic acid, sulfanilic acid, picolinic or nicotinic acid.

Preferred radicals D are, e.g. aryl-C_1-C_3alkyl radicals as for example phenyl-C_1-C_3alkyl, hydroxyphenyl-C_1-C_3alkyl, imidazolyl-C_1-C_3alkyl or naphthyl-C_1-C_3alkyl. More preferred, radical D is the side chain of an aromatic amino acid like Trp, Phe, Tyr and His, wherein the side chain of Trp is especially preferred.

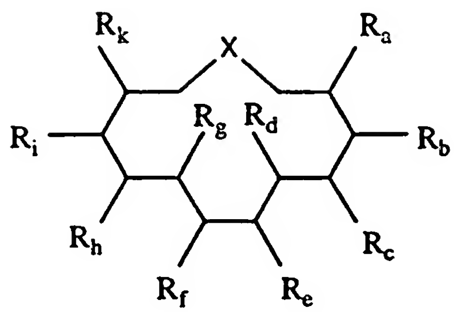
The radical E is for example an amino group optionally substituted by one or two methyl, ethyl, propyl, butyl, aminomethyl or aminoethyl; or a five- or six-membered one or two nitrogen containing heterocyclic radical linked via a nitrogen atom to the cyclic radical A, e.g. piperidyl, 2-oxo-pyrrolidyl or piperazyl. The radical E can also be NH_2 substituted by an acyl radical of a naturally occurring amino acid or an acyl radical of a peptide containing 2-5 naturally occurring amino acids.

The $(CH_2)_x$ and $(CH_2)_y$-groups are for example unbranched chains such as methylene, ethylene, propylene and butylene.

The ring forming atoms of the cyclic radical A are unsubstituted or substituted by small substituents like C_1-C_4alkyl groups. Preferably said ring forming atoms are unsubstituted or substituted by methyl or ethyl.

The basic compounds of formula (1) can form acid addition salts. Pharmaceutical acceptable acid addition salts are, for example, salts of compounds of the formula (1) with suitable mineral acids, such as hydrohalic acids, sulfuric acid or phosphoric acid, e.g. hydrochlorides, hydrobromides, sulfates, hydrogen sulfates or phosphates, salts with suitable aliphatic or aromatic sulfonic acids or N-substituted sulfamic acids, e.g. methane-sulfonates, benzenesulfonates, p-toluenesulfonates or N-cyclohexylsulfamates (cyclamates), or salts with organic acids, such as lower alkanecarboxylic acids or optionally unsaturated or hydroxylated aliphatic dicarboxylic acids, e.g. acetates, oxalates, maleates, malonates, fumarates, malates or citrates. If possible, the formation of an inner salt between an amino group and a carboxyl group of a compound of the formula (1) is preferred.

Preferred is a cyclic compound of formula (1a)



(1a)

Wherein up to 6, preferred 3, of the ring forming C-atoms can be replaced by oxygen, sulfur or nitrogen, which is preferred; and the ring forming atoms can additionally be substituted by up to 6, preferred up to 3, C_1-C_4alkyl, oxo, hydroxy, sulfo and amino radicals, wherein methyl, ethyl or oxo is preferred.

X = C_1-C_4alkylene or C_1-C_4alkenylene wherein up to 2 C-atoms, prefered is one, can be replaced by oxygen, sulfur, disulfide, sulfoxy, sulfonyl or nitrogene, preferred is oxigen, sufur and disulfide. The C_1-C_4alkylene or alkenylene group can be substituted by methyl, ethyl, oxo or amino. Examples are CH_2, C_2H_4, C_2H_2, $CH(CH_3)$-CH_2, S, S-CH_2, CH_2-S, CH_2-S-S, S-S-CH_2, S-S, S=O, O=S=O, NH, NH-CO, CO-NH, O, O-CH_2, CH_2-O, Se; preferred is CH_2, C_2H_4, S, S-CH_2, CH_2-S, CH_2-S-S, S-S, NH, NH-CO, CO-NH, O, O-CH_2, CH_2-O, Se; and most preferred is CH_2, CH_2-CH_2, S, S-CH_2, CH_2-S, S-S-CH_2, S-S, NH-CO, CO-NH, O-CH_2, O-CH_2;

R_a or R_b = radical of a naturally occurring amino acid or a radical of a peptide containing 2-5 naturally occurring amino acids, wherein the first or second amino acid after the ring forming atom is an amino acid carrying an aromatic side chain, e.g. tryptophane, phenylalanine, tyrosine or histidine; or C_1-C_7alkyl-aryl, wherein the C-atoms of the C_1-C_7alkyl spacer group can be replaced by up to 3 nitrogen or oxygen, wherein nitrogen is preferred. The spacer group can be substituted by oxo; C_1-C_4alkyl wherein methyl and ethyl are preferred; amino, oxo and carboxy.

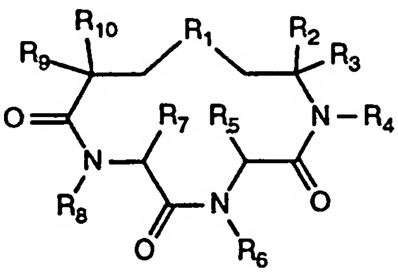
R_c, R_d or R_e = hydroxy or C_1-C_4alkyl substituted with hydroxy; preferred hydroxy, hydroxymethyl and hydroxyethyl; more preferred hydroxy and hydroxymethyl; and most preferred hydroxymethyl;

R_f, R_g or R_h = C_2-C_5alkyl substituted with -C(=NH)NH_2 or -NH-C(=NH)NH_2; preferred -CH_2-CH_2-CH_2-NH-C(=NH)NH_2;

R_i or R_k = NH_2; NH_2 substituted with C_1-C_4alkyl or amino-C_1-C_4alkyl; C_1-C_4alkyl substituted with one or more, especially one or two, amino groups; a five- or six-membered one or two nitrogen containing heterocyclic radical; or a radical of a naturally occurring amino acid or of a peptide containing 2-5 naturally occurring

amino acids;

A more preferred cyclic compound is a heterocyclic compound of the formula (2)



(2)

wherein

R_1 = CH_2, C_2H_4, C_2H_2, $CH(CH_3)$-CH_2, S, CH_2-S, CH_2-S-S, S-S-CH_2, S-S, S=O, O=S=O, NH, NH-CO, O, O-CH_2, CH_2-O, Se; preferred is CH_2, C_2H_4, S, CH_2-S, CH_2-S-S, S-S, NH, NH-CO, O, O-CH_2, CH_2-O, Se; and most preferred is CH_2, CH_2-CH_2, S, CH_2-S, CH_2-S-S, S-S, NH-CO, O-CH_2;

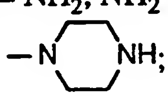
R_2 = -C_1-C_6alkyl-aryl or -CO-NH-CH(C_1-C_3alkyl-aryl)R_{11}; preferred is -C_1-C_6alkyl-R_{13} or -CO-NH-CH(C_1-C_3alkyl-R_{13})R_{11}; and most preferred is -CO-NH-CH(C_1-C_3alkyl-R_{13})R_{11};

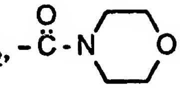
R_3, R_4, R_6 and R_9 independently of each other are hydrogen or C_1-C_4alkyl; preferred hydrogen, methyl or ethyl; more preferred hydrogen or methyl; and most preferred hydrogen;

R_5 = hydroxy or C_1-C_4alkyl substituted with hydroxy; preferred hydroxy, hydroxymethyl and hydroxyethyl; more preferred hydroxy and hydroxymethyl; and most preferred hydroxymethyl;

one of the residues R_7 and R_8 is C_2-C_5alkyl substituted with -C(=NH)NH_2 or -NH-C(=NH)NH_2; preferred -CH_2-CH_2-CH_2-NH-C(=NH)NH_2; and the other residues is hydrogen or C_1-C_4alkyl; preferred hydrogen or methyl; and most preferred hydrogen;

R_{10} = NH_2; NH_2 substituted with C_1-C_4alkyl; C_1-C_4alkyl substituted with NH_2;

; -NH-CO-CH_2-NH_2 or -NH-CH_2-CH_2-NH_2 unsubstituted or substituted with methyl or ethyl; preferred is NH_2; NH_2 substituted with C_1-C_2alkyl; C_1-C_2alkyl substituted with NH_2; -NH-CO-CH_2-NH_2 or -NH-CO-CH(CH_3)-NH_2; more preferred NH_2; -NH-CH_3; -CH_2-NH_2; -CH_2-CH_2-NH_2; -NH-CO-CH_2-NH_2 or -NH-CO-CH(CH_3)-NH_2; and most preferred NH_2; -NH-CO-CH_2-NH_2 or -NH-CO-CH(CH_3)-NH_2;

R_{11} = hydrogen, -COOH, -CONH$_2$,  or -CO-R_{12}; preferred hydrogen, -COOH, -CONH$_2$ or -CO-R_{12}; and most preferred hydrogen, -COOH or -CONH$_2$;

R_{12} = a radical of a naturally occurring amino acid or a radical of a peptide containing 2-5 naturally occurring amino acids; preferred is the radical of a naturally occurring amino acid; wherein the amino acid radical or the peptide radical is preferably bound via an amide linkage;

R_{13} = phenyl, hydroxyphenyl, naphthyl, indolyl, pyridyl, quinolyl, isoquinolyl or pyrrolyl; preferred phenyl, hydroxyphenyl or indolyl; and most preferred indolyl.

In a further preferred compound of the formula (2)

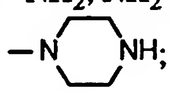
R_1 = CH_2, C_2H_4, S, CH_2-S, CH_2-S-S, S-S, NH, NH-CO, O, O-CH_2, H_2C-O, Se;

R_2 = -C_1-C_6alkyl-R_{13} or -CO-NH-CH(C_1-C_3alkyl-R_{13})R_{11};

R_3, R_4, R_6 and R_9 independently of each other are hydrogen, methyl or ethyl;

R_5 = hydroxy or C_1-C_3alkyl substituted with hydroxy;

one of the residues R_7 and R_8 is C_2-C_5alkyl substituted with -C(=NH)NH$_2$ or -NH-C(=NH)NH$_2$; and the other residue is hydrogen, methyl or ethyl;

R_{10} = NH$_2$; NH$_2$ substituted with C_1-C_4alkyl; C_1-C_4alkyl substituted with NH$_2$; ; -NH-CO-CH$_2$-NH$_2$; -NH-CO-CH(CH$_3$)-NH$_2$ or -NH-CH$_2$-CH$_2$-NH$_2$;

R_{11} = hydrogen, -COOH, -CONH$_2$ or -CO-R_{12};

R_{12} = a radical of a naturally occurring amino acid;

R_{13} = phenyl, hydroxyphenyl, naphthyl, indolyl, pyridyl, quinolyl, isoquinolyl or pyrrolyl.

More preferred is a compound of the formula (2) wherein

R_1 = CH_2, CH_2-CH_2, S, CH_2-S, CH_2-S-S, S-S, NH-CO, O, O-CH_2, CH_2-O;

R_2 = -C_3-C_6alkyl-R_{13} or -CO-NH-CH(C_1-C_3alkyl-R_{13})R_{11};

R_3, R_4, R_6 and R_9 independently of each other are hydrogen, methyl or ethyl;

R_5 = hydroxy, hydroxymethyl or hydroxyethyl;

one of the residues R_7 and R_8 is C_2-C_5alkyl substituted with -C(=NH)NH$_2$ or -NH-C(=NH)NH$_2$; and the other residue is hydrogen, methyl or ethyl;

R_{10} = NH$_2$; NH$_2$ substituted with C_1-C_2alkyl; C_1-C_2alkyl substituted with NH$_2$; -NH-CO-CH$_2$-NH$_2$ or -NH-CO-CH(CH$_3$)-NH$_2$;

R_{11} = hydrogen, -COOH, -CONH$_2$ or -CO-R_{12};

R_{12} = a radical of a naturally occurring amino acid;

R_{13} = phenyl, hydroxyphenyl or indolyl.

Most preferred is a compound of the formula (2) wherein

R_1 = CH_2, CH_2-CH_2, S, CH_2-S, CH_2-S-S, S-S, NH-CO, O, O-CH_2, CH_2-O;

R_2 = -C_3-C_6alkyl-R_{13} or -CO-NH-CH(C_1-C_3alkyl-R_{13})R_{11};

R_3, R_4, R_6 and R_9 independently of each other are hydrogen or methyl;

R_5 = hydroxy, hydroxymethyl or hydroxyethyl;

one of the residues R_7 and R_8 is C_2-C_5alkyl substituted with -C(=NH)NH_2 or -NH-C(=NH)NH_2; and the other residue is hydrogen, methyl or ethyl;

R_{10} = NH_2; -NH-CH_3, -NH-CH_2-CH_3; -CH_2-NH_2; -CH_2-CH_2-NH_2; -NH-CO-CH_2-NH_2 or -NH-CO-CH(CH_3)-NH_2;

R_{11} = hydrogen, -COOH, -CONH_2 or -CO-R_{12};

R_{12} = a radical of a naturally occurring amino acid;

R_{13} = phenyl, hydroxyphenyl or indolyl;

a compound of the formula (2) wherein

R_1 = CH_2, CH_2-CH_2, S, CH_2-S, CH_2-S-S, S-S, NH-CO, O-CH_2;

R_2 = -CO-NH-CH(C_1-C_3alkyl-R_{13})R_{11};

R_3 = hydrogen or methyl;

R_4, R_6 and R_9 are hydrogen;

R_5 = hydroxy, hydroxymethyl or hydroxyethyl;

one of the residues R_7 and R_8 is -CH_2-CH_2-CH_2-NH-C(=NH)NH_2; and the other residue is hydrogen;

R_{10} = NH_2; -NH-CH_3; -CH_2-NH_2; -CH_2-CH_2-NH_2; -NH-CO-CH_2-NH_2 or -NH-CO-CH(CH_3)-NH_2;

R_{11} = hydrogen, -COOH or -CONH_2;

R_{13} = phenyl, hydroxyphenyl or indolyl;

and a compound of the formula (2) wherein

R_1 = CH_2, CH_2-CH_2, S, CH_2-S, CH_2-S-S, S-S, NH-CO, O-CH_2;

R_2 = -CO-NH-CH(CH_2-R_{13})R_{11};

R_3 = hydrogen or methyl;

R_4, R_6 and R_9 are hydrogen;

R_5 = hydroxy or hydroxymethyl;

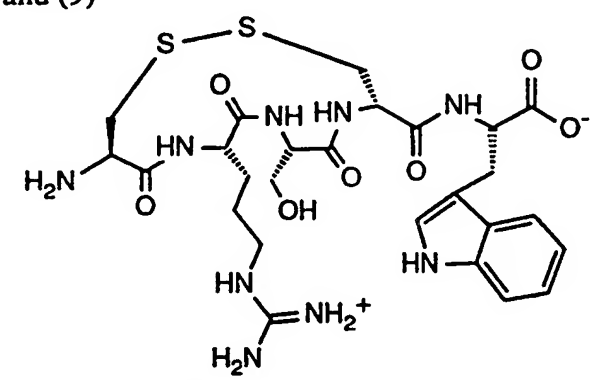
one of the residues R_7 and R_8 is -CH_2-CH_2-CH_2-NH-C(=NH)NH_2; and the other residue is hydrogen;

R_{10} = NH_2; -NH-CO-CH_2-NH_2 or -NH-CO-CH(CH_3)-NH_2; wherein NH_2 is most preferred;

R_{11} = hydrogen, -COOH or -CONH$_2$;

R_{13} = indolyl;

Examples of the compounds according to the invention are those of formula (3), (4), (5), (6), (7), (8) and (9)



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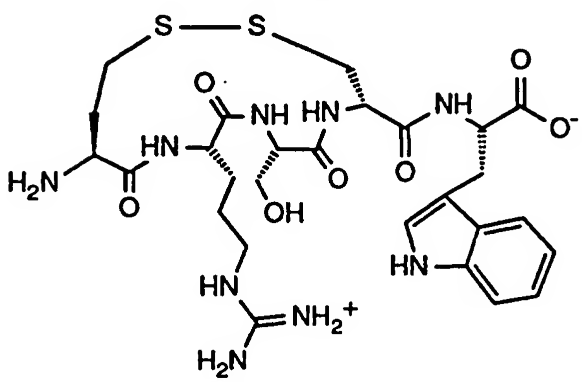

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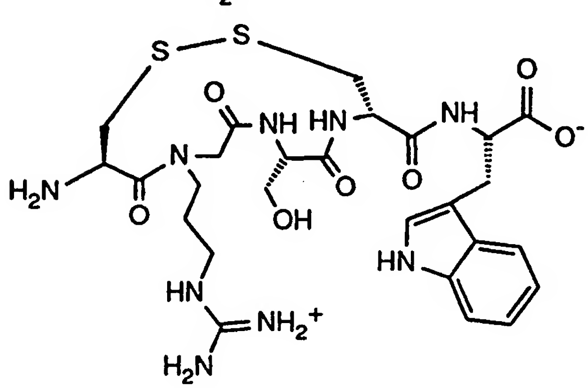

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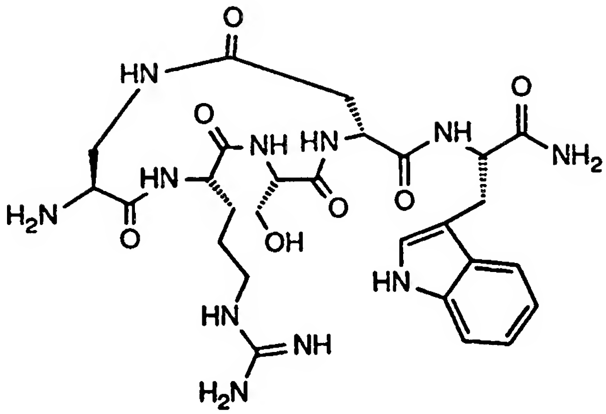

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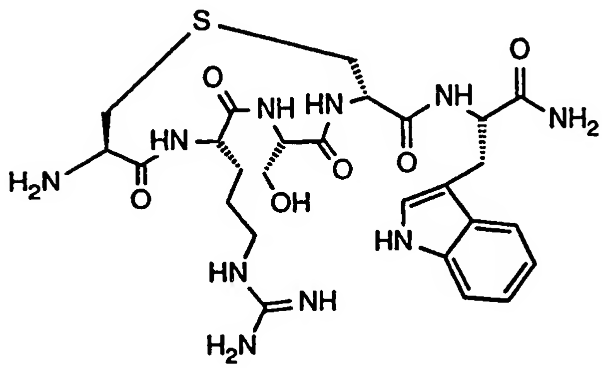

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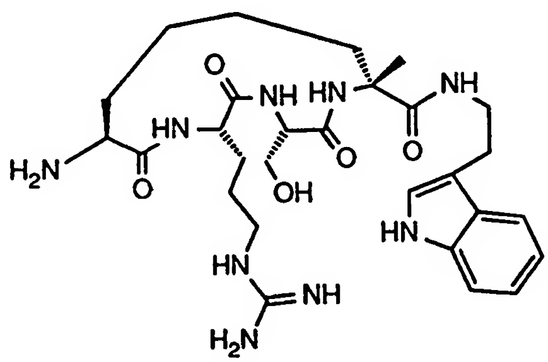

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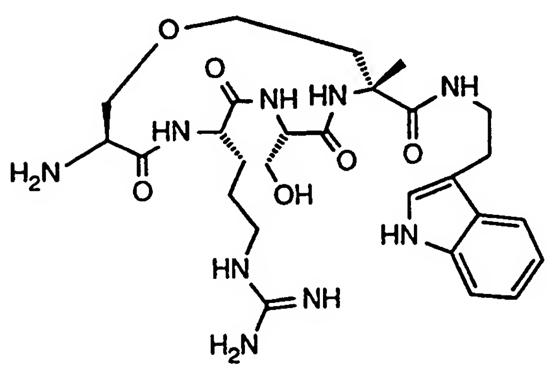
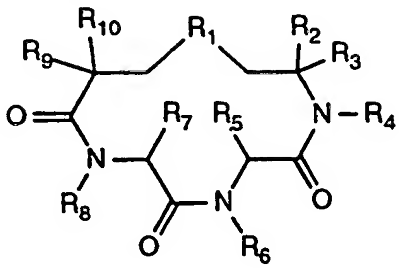


(9)

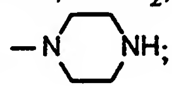
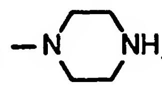
Another preferred cyclic compound is a heterocyclic compound of the formula (2)



(2)

wherein

R_1 = CH_2, C_2H_4, C_2H_2, $CH(CH_3)$-CH_2, S, CH_2-S, CH_2-S-S, S-S-CH_2, S-S, S=O, O=S=O, NH, NH-CO, O, O-CH_2, CH_2-O, Se; preferred is CH_2, C_2H_4, S, CH_2-S, CH_2-S-S, S-S, NH, NH-CO, O, O-CH_2, CH_2-O, Se; and most preferred is CH_2, CH_2-CH_2, S, CH_2-S, S-S, S-S-CH_2, NH-CO, O-CH_2;

R_2 = NH_2, C_1-C_4alkyl substituted with NH_2, ; or -CO-R_{12}-NH_2; preferred NH_2, C_1-C_4alkyl substituted with NH_2, , -CO-NH-CH_2-CO-NH_2, -CO-NH-$CH(CH_3)$-CO-NH_2 or -CO-NH-CH_2-CH_2-NH_2; more preferred NH_2, -CH_2-NH_2, -CH_2-CH_2-NH_2, -CO-NH-CH_2-CO-NH_2, -CO-NH-$CH(CH_3)$-CO-NH_2 or -CO-NH-CH_2-CH_2-NH_2; and most preferred -CH_2-NH_2, -CO-NH-CH_2-CO-NH_2 or -CO-NH-CH_2-CH_2-NH_2;

R_3, R_4, R_8 and R_9 independently of each other are hydrogen or C_1-C_4alkyl; preferred hydrogen, methyl or ethyl; more preferred hydrogen or methyl; and most preferred hydrogen;

one of the residues R_5 and R_6 is C_2-C_5alkyl substituted with -C(=NH)NH_2 or -NH-C(=NH)NH_2; preferred -CH_2-CH_2-CH_2-NH-C(=NH)NH_2; and the other residue is hydrogen or C_1-C_4alkyl; preferred hydrogen or methyl; and most preferred hydrogen;

R_7 = hydroxy or C_1-C_4alkyl substituted with hydroxy; preferred hydroxy, hydroxymethyl and hydroxyethyl; more preferred hydroxy and hydroxymethyl; and most preferred hydroxymethyl;

R_{10} = -NH-C_1-C_6alkyl-aryl or -NH-CO-CH(C_1-C_3alkyl-aryl)R_{11}; preferred -NH-C_1-C_6alkyl-R_{13} or -NH-CO-CH(C_1-C_3alkyl-R_{13})R_{11}; and most preferred -NH-CO-CH(C_1-C_3alkyl-R_{13})R_{11};

R_{11} = hydrogen, NH_2, -NH-CO(C_1-C_4alkyl) or -NH-R_{12}; preferred hydrogen, NH_2, -NH-$COCH_3$, -NH-$COCH_2$-CH_3 or -NH-R_{12}; and most preferred hydrogen, NH_2 or -NH-$COCH_3$;

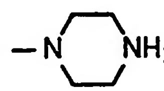
R_{12} = a radical of a naturally occurring amino acid or a radical of a peptide containing

2-5 naturally occurring amino acids; preferred is the radical of a naturally occurring amino acid; wherein the amino acid radical or the peptide radical is preferably bound via an amide linkage;

R_{13} = phenyl, hydroxyphenyl, naphthyl, indolyl, pyridyl, quinolyl, isoquinolyl or pyrrolyl; preferred phenyl, hydroxyphenyl or indolyl; and most preferred indolyl.

In a further preferred compound of the formula (2) as described above

R_1 = CH_2, C_2H_4, S, CH_2-S, CH_2-S-S, S-S-CH_2, S-S, NH, NH-CO, O, Se, O-CH_2, CH_2-O;

R_2 = NH_2; C_1-C_4alkyl substituted with NH_2; ; -CO-NH-CH_2-CO-NH_2; -CO-NH-CH(CH_3)-CO-NH_2 or -CO-NH-CH_2-CH_2-NH_2;

R_3, R_4, R_8 and R_9 independently of each other are hydrogen or methyl or ethyl;

one of the residues R_5 and R_6 is C_2-C_5alkyl substituted with -C(=NH)NH_2 or -NH-C(=NH)NH_2; and the other residue is hydrogen, methyl or ethyl;

R_7 = hydroxy or C_1-C_3alkyl substituted with hydroxy;

R_{10} = -NH-C_1-C_6alkyl-R_{13} or -NH-CO-CH(C_1-C_3alkyl-R_{13})R_{11};

R_{11} = hydrogen, NH_2, -NH-$COCH_3$, -NH-$COCH_2$-CH_3 or -NH-R_{12};

R_{12} = a radical of a naturally occurring amino acid;

R_{13} = phenyl, hydroxyphenyl, naphthyl, indolyl, pyridyl, quinolyl, isoquinolyl or pyrrolyl.

More preferred is a compound of the formula (2) wherein

R_1 = CH_2, CH_2-CH_2, S, CH_2-S, CH_2-S-S, S-S-CH_2, S-S, NH-CO, O, O-CH_2, CH_2-O;

R_2 = NH_2; C_1-C_4alkyl substituted with NH_2; ; -CO-NH-CH_2-CO-NH_2; -CO-NH-CH(CH_3)-CO-NH_2 or -CO-NH-CH_2-CH_2-NH_2;

R_3, R_4, R_8 and R_9 independently of each other are hydrogen, methyl or ethyl;

one of the residues R_5 and R_6 is C_2-C_5alkyl substituted with -C(=NH)NH_2 or -NH-C(=NH)NH_2; and the other residue is hydrogen, methyl or ethyl;

R_7 = hydroxy, hydroxymethyl or hydroxyethyl;

R_{10} = -NH-C_3-C_6alkyl-R_{13} or -NH-CO-CH(C_1-C_3alkyl-R_{13})R_{11};

R_{11} = hydrogen, NH_2, -NH-$COCH_3$, -NH-$COCH_2$-CH_3 or -NH-R_{12};

R_{12} = a radical of a naturally occurring amino acid;

R_{13} = phenyl, hydroxyphenyl, naphthyl, indolyl, pyridyl, quinolyl, isoquinolyl or pyrrolyl.

Most preferred is a compound of the formula (2) wherein

R_1 = CH_2, CH_2-CH_2, S, CH_2-S, CH_2-S-S, S-S-CH_2, S-S, NH-CO, O, O-CH_2, CH_2-O;
R_2 = NH_2; -CH_2-NH_2; -CH_2-CH_2-NH_2; -CO-NH-CH_2-CO-NH_2;
-CO-NH-CH(CH_3)-CO-NH_2; or -CO-NH-CH_2-CH_2-NH_2;
R_3, R_4, R_8 and R_9 independently of each other are hydrogen or methyl;
one of the residues R_5 and $R_6$is hydrogen or methyl and the other is C_2-C_4alkyl
substituted with -C(=NH)NH_2 or -NH-C(=NH_2)NH_2;
R_7 = hydroxy; hydroxymethyl or hydroxyethyl;
R_{10} = -NH-C_3-C_6alkyl-R_{13} or -NH-CO-CH(C_1-C_3alkyl-R_{13})R_{11};
R_{11} = hydrogen, NH_2, -NH-$COCH_3$ or -NH-R_{12};
R_{12} = a radical of a naturally occurring amino acid;
R_{13} = phenyl, hydroxyphenyl or indolyl;

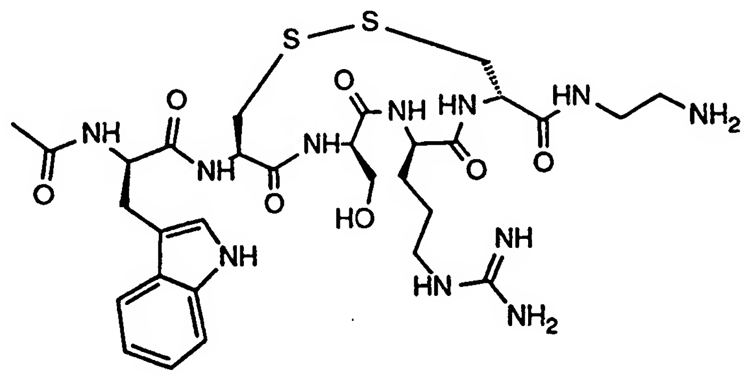
a compound of the formula (2) wherein

R_1 = S, CH_2-CH_2, CH_2-S, S-S, CH_2-S-S, NH-CO, O-CH_2, CH_2-O;
R_2 = NH_2, -CH_2-NH_2, -CH_2-CH_2-NH_2, -CO-NH-CH_2-CO-NH_2,
-CO-NH-CH(CH_3)-CO-NH_2 or -CO-NH-CH_2-CH_2-NH_2;
R_3, R_4 and R_8 = hydrogen;
one of the residues R_5 and $R_6$is hydrogen and the other is
-CH_2-CH_2-CH_2-NH-C(=NH)NH_2;
R_7 = hydroxy, hydroxymethyl or hydroxyethyl;
R_9 = hydrogen or methyl;
R_{10} = -NH-CO-CH(C_1-C_3alkyl-R_{13})R_{11};
R_{11} = hydrogen, NH_2 or -NH-$COCH_3$;
R_{13} = phenyl, hydroxyphenyl or indolyl;

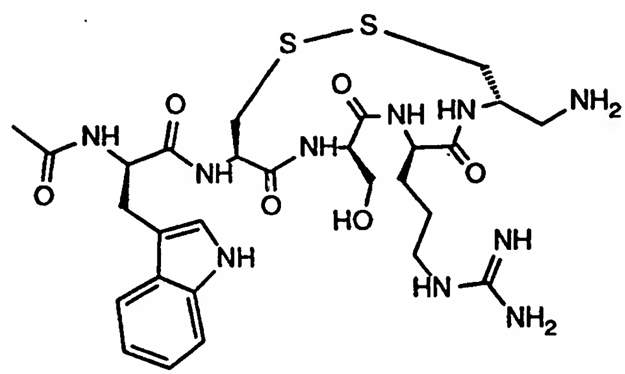
and a compound of the formula (2) wherein

R_1 = S, CH_2-S, S-S, CH_2-S-S, NH-CO;
R_2 = -CH_2-NH_2, -CO-NH-CH_2-CO-NH_2 or -CO-NH-CH_2-CH_2-NH_2;
R_3, R_4, R_6 and R_8 = hydrogen;
R_5 = -CH_2-CH_2-CH_2-NH-C(=NH)NH_2;
R_7 = hydroxy or hydroxymethyl;
R_9 = hydrogen or methyl;
R_{10} = -NH-CO-CH(CH_2-R_{13})R_{11};
R_{11} = NH_2 or -NH-$COCH_3$;
R_{13} = indolyl.

Examples of the compounds according to the invention are those of formula (10) and (11)



(10)



(11)

The compounds according to the invention are synthesized in a manner known per se.

The synthesis of the compounds depends on the cyclic radical used. Crown ethers, steroids and porphyrines are commercially available or can be isolated by standard methods. The functional groups of the compounds can be introduced by conventional methods of organic chemistry. Cyclic peptides consisting of various amino acids, amino acid analogs or modified amino acids can be synthesized in a manner known per se.

The synthesis of modified amino acids is described, for example, in R.M. Williams, Synthesis of optically active α-amino acids, Pergamon Press 1989; J.P. Greenstein and M. Winitz, Chemistry of the Amino Acids, Whiley & Sons INC., 1961; R.J. Simon et al. Proc. Natl. Acad. Sci. USA (1992), 89, 9367-9371; E. Altmann et al. Helv. Chim. Acta (1991), 74, 800-806 and M. Rodrigez et al. Tetrahedron Lett. (1991), 32, 923-926.

The process for the production of a cyclic peptide according to the invention comprises, for example, reacting an amide bond forming first fragment of a compound of formula (1)

with a second amide bond forming fragment of a compound of formula (1), said first fragment and said second fragment being complementary to one another such that an amide bond is formed between said first and second fragments to result in said compound of formula (1), one of said first and second fragments containing a reactive free carboxy group and sulfoxy group, respectively, or a reactive carboxylic acid or sulfonic acid derivative thereof, and the other of said first and second fragments containing a free amino group or a reactive derivative thereof, wherein free functional groups in the mentioned fragments, with the exception of the two groups participating in the reaction, are, if necessary, in protected form, and removing protecting groups which may be present, and, if desired, converting a salt obtainable in accordance with the process into the free compound and/or converting a free compound obtainable in accordance with the process into a salt. Suitable synthetic methods are described, for example, in M. Bodanszky, Peptide Chemistry, Springer Verlag, Berlin 1988, and in E. Atherton and R.C. Sheppard, Solid Phase Peptide Synthesis, IRL press, Oxford, 1989.

Reactive carboxylic acid and sulfonic acid derivatives are especially reactive activated esters or reactive anhydrides, and also reactive cyclic amides; as mentioned, reactive carboxylic acid derivatives can be formed in situ.

Reactive carboxylic acid and sulfonic acid derivatives are especially reactive activated esters or reactive anhydrides, and also reactive cyclic amides; as mentioned, reactive carboxylic acid derivatives can be formed in situ, e.g. by the use of a suitable condensing agent.

Activated esters of acids are especially esters that are unsaturated at the linking carbon atom of the esterifying radical, for example of the vinyl ester type, such as true vinyl esters (which can be obtained, for example, by the transesterification of a corresponding ester with vinyl acetate; activated vinyl ester method), carbamoylvinyl esters (which can be obtained, for example, by treating the corresponding acid with an isoxazolium reagent; 1,2-oxazolium or Woodward method), or 1-lower alkoxyvinyl esters (which can be obtained, for example, by treating the corresponding acid with a lower alkoxyacetylene; ethoxyacetylene method), or esters of the amidino type, such as N,N'-disubstituted amidino esters (which can be obtained, for example, by treating the corresponding acid with a suitable N,N'-disubstituted carbodiimide, for example N,N'-diisopropyl- or N,N'-dicyclohexyl-carbodiimide; carbodiimide method), or N,N-disubstituted amidino esters (which can be obtained, for example, by treating the corresponding acid with an

N,N-disubstituted cyanamide; cyanamide method). Activated esters are also, for example, suitable aryl esters, especially phenyl esters suitably substituted by electronattracting substituents (which esters can be obtained, for example, by treating the corresponding acid with a suitably substituted phenol, for example 4-nitrophenol, 4-methylsulfonylphenol, 2,4,5-trichlorophenol, 2,3,4,5,6-pentachlorophenol, pentafluorophenol or 4-phenyldiazophenol, in the presence of a condensing agent, such as N,N'-dicyclohexylcarbodiimide; activated aryl esters method) cyanomethyl esters (which can be obtained, for example, by treating the corresponding acid with chloroacetonitrile in the presence of a base; cyanomethyl esters method), suitable thioesters, especially phenylthio esters that are unsubstituted or substituted, for example, by nitro (which can be obtained, for example, by treating the corresponding acid with a thiophenol that is unsubstituted or substituted, for example, by nitro, inter alia with the aid of the anhydride or carbodiimide method; activated thiol esters method), or amino or amido esters (which can be obtained, for example, by treating the corresponding acid with an N-hydroxyamino or N-hydroxyamido compound, for example N-hydroxysuccinimide, N-hydroxypiperidine, N-hydroxyphthalimide or 1-hydroxy-1H-benzotriazole, for example according to the anhydride or carbodiimide method; activated N-hydroxy esters method).

Reactive acid anhydrides may be symmetrical or preferably mixed anhydrides of these acids, for example anhydrides with inorganic acids, such as acid halides, especially acid chlorides (which can be obtained, for example, by treating the corresponding acid, including sulfonic acid, with a suitable halogenating agent, such as thionyl chloride, phosphorus pentachloride or oxalyl chloride; acid chloride method); azides (which can be obtained, for example, from an acid ester by way of the corresponding hydrazide and treatment thereof with nitrous acid; azide method), anhydrides with carbonic acid semi-derivatives, such as with corresponding esters, for example carbonic acid lower alkyl semi-esters (which can be obtained, for example, by treating the corresponding acid with haloformic, such as chloroformic, acid lower alkyl esters or with a 1-lower alkoxycarbonyl-2-lower alkoxy-1,2-dihydroquinoline, for example 1-lower alkoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; mixed O-alkylcarbonic acid anhydrides method), or anhydrides with dihalogenated, especially dichlorinated, phosphoric acid (which can be obtained, for example, by treating the corresponding acid with phosphorus oxychloride; phosphorus oxychloride method), or anhydrides with organic acids, such as mixed anhydrides with organic carboxylic acids (which can be obtained, for example, by treating the corresponding acid with an unsubstituted or substituted lower alkanecarboxylic or phenyl-lower alkanecarboxylic acid halide, for example phenylacetic acid, pivalic acid or

trifluoroacetic acid chloride; mixed carboxylic acid anhydrides method) or with organic sulfonic acids (which can be obtained, for example, by treating a salt, such as an alkali metal salt, of the corresponding acid with a suitable organic sulfonic acid halide, such as a lower alkanesulfonic or arylsulfonic acid chloride, for example methane- or p-toluene-sulfonic acid chloride; mixed sulfonic acid anhydrides method), as well as symmetrical anhydrides (which can be obtained, for example, by condensation of the corresponding acid in the presence of a carbodiimide or of 1-diethylaminopropyne; symmetrical anhydrides method).

Suitable cyclic amides are especially amides with five-membered diazacycles of aromatic character, such as amides with imidazoles, for example imidazole (which can be obtained, for example, by treating the corresponding acid with N,N'-carbonyldiimidazole; imidazolide method) or pyrazoles, for example 3,5-dimethylpyrazole (which can be obtained, for example, by way of the acid hydrazide by treatment with acetylacetone; pyrazolide method).

As mentioned, the carboxylic acid derivatives can also be formed in situ. For example, N,N'-disubstituted amidino esters can be formed in situ by reacting a mixture of the complementary fragment having the free amino group and the peptide fragment having a free carboxy group in the presence of a suitable N,N-disubstituted carbodiimide, for example N,N-diisopropyl- or N,N'-dicyclohexyl-carbodiimide. Further, amino or amido esters of acids can be formed in the presence of the amine to be acylated by reacting a mixture of the corresponding acid and amino starting materials in the presence of an N,N'-disubstituted carbodiimide, for example N,N'-dicyclohexyl- or N,N'-diisopropyl-carbodiimide, and in the presence of an N-hydroxyamine or N-hydroxyamide, for example N-hydroxysuccinimide, optionally in the presence of a suitable base, for example 4-dimethylaminopyridine.

Alternatively, the process according to the invention can also be carried out by reacting a fragment having a free carboxy group with the complementary fragment in which the amino group is present in reactive form; the amino group can be activated, for example, by reaction with a phosphite, for example diethyl chlorophosphite, 1,1-phenylene chlorophosphite, ethyl dichlorophosphite, ethylene chlorophosphite or tetraethyl pyrophosphite, or with a suitable silylation agent, such as an organic halosilane, for example trimethylchlorosilane. The amino group can also be activated by bonding to halocarbonyl, for example chlorocarbonyl, or can be activated in the form of an isocyanate group.

Functional groups in the mentioned fragments which, if they are not to participate in the reaction, are advantageously in protected form, are especially carboxy, amino and hydroxy groups, and also carbamoyl and guanidino groups.

Protecting groups and the manner in which they are introduced and removed are described, for example, in "Protective Groups in Organic Chemistry", Plenum Press, London, New York 1973, in "Methoden der organischen Chemie", Houben-Weyl, 4th Edition, Vol. 15/1, Georg-Thieme-Verlag, Stuttgart 1974, and in Th. W. Greene, "Protective Groups in Organic Synthesis", John Wiley & Sons, New York 1981. It is characteristic of protecting groups that they can be readily removed, that is to say without undesired secondary reactions taking place, for example by solvolysis, reduction or photolysis.

Hydroxy-protecting groups are, for example, acyl radicals, such as unsubstituted or substituted, for example halo-substituted, lower alkanoyl, such as 2,2-dichloroacetyl, or acyl radicals of carbonic acid semi-esters, especially tert.-butoxycarbonyl, unsubstituted or substituted benzyloxycarbonyl, for example 4-nitrobenzyloxycarbonyl, or diphenylmethoxycarbonyl, or 2-halo-lower alkoxycarbonyl, such as 2,2,2-trichloroethoxycarbonyl, or formyl. Other hydroxy-protecting groups are, for example, suitable etherifying groups, such as trityl, tert.-lower alkyl, for example tert.-butyl, 2-oxa- or 2-thia-aliphatic or -cycloaliphatic hydrocarbon radicals, especially 1-lower alkoxy-lower alkyl or 1-lower alkylthio-lower alkyl, for example methoxymethyl, 1-methoxyethyl, 1-ethoxyethyl, methylthiomethyl, 1-methylthioethyl or 1-ethylthioethyl, or 2-oxa- or 2-thia-cycloalkyl having 5 or 6 ring atoms, for example 2-tetrahydrofuryl or 2-tetrahydropyranyl or corresponding thia analogues, and also unsubstituted or substituted 1-phenyl-lower alkyl, such as unsubstituted or substituted benzyl or diphenylmethyl, there being suitable as substituents of phenyl radicals, for example, halogen, such as chlorine, lower alkoxy, such as methoxy, and/or nitro. Further hydroxy-protecting groups are also organic silyl or stannyl radicals that preferably contain lower alkyl, especially methyl, and/or aryl, for example phenyl, as substituents, especially tri-lower alkylsilyl, especially trimethylsilyl, and also dimethyl-tert.-butyl-silyl, or correspondingly substituted stannyl, for example tri-n-butylstannyl.

Thiol groups are protected in analogous manner, for example, with trityl, acetamidomethyl or tert.-butyl.

Carboxy groups are preferably protected in esterified form, such ester groupings being readily cleavable under mild conditions. Carboxy groups protected in this manner contain as esterifying groups especially lower alkyl groups that are branched at the 1-position or suitably substituted at the 1- or 2-position. Preferred carboxy groups in esterified form are, inter alia, tert.-lower alkoxycarbonyl, for example tert.-butoxycarbonyl, α-aryl-lower alkoxycarbonyl having one or two aryl radicals, these being phenyl radicals that are unsubstituted or substituted, for example, by lower alkyl, such as tert.-lower alkyl, for example tert.-butyl, lower alkoxy, such as methoxy, hydroxy, halogen, for example chlorine, nitro and/or by phenyl, such as benzyloxycarbonyl that is unsubstituted or substituted, for example, as mentioned above, for example 4-methoxybenzyloxycarbonyl or 4-nitrobenzyloxycarbonyl, biphenylyl-lower alkoxycarbonyl in which biphenylyl substitutes the α-position, for example 2-(p-biphenylyl)-2-propoxycarbonyl, or diphenyl-methoxycarbonyl that is unsubstituted or substituted, for example, as mentioned above, for example diphenylmethoxycarbonyl or di-(4-methoxyphenyl)-methoxycarbonyl, 1-lower alkoxy-lower alkoxycarbonyl, such as methoxymethoxycarbonyl, 1-methoxyethoxycarbonyl or 1-ethoxymethoxycarbonyl, 2,2-diaryl-ethoxycarbonyl in which aryl is phenyl that is unsubstituted or substituted, for example, by nitro, such as 4-nitrophenyl, such as 2,2-di-(4-nitrophenyl)-ethoxycarbonyl, wherein the two aryl, for example phenyl, radicals may also be bonded to one another, for example 2-(9-fluorenyl)-ethoxycarbonyl, 1-lower alkylthio-lower alkoxycarbonyl, such as 1-methylthiomethoxycarbonyl or 1-ethylthio-ethoxycarbonyl, aroylmethoxycarbonyl in which the aroyl group is benzoyl that is unsubstituted or substituted, for example, by halogen, such as bromine, for example phenacyloxycarbonyl, 2-halo-lower alkoxycarbonyl, for example 2,2,2-trichloroethoxy-carbonyl, 2-bromoethoxycarbonyl or 2-iodoethoxycarbonyl, or 2-(trisubstituted silyl)-ethoxycarbonyl in which each of the substituents, independently of the others, represents an aliphatic, araliphatic, cycloaliphatic or aromatic hydrocarbon radical that is unsubstituted or substituted, for example, by lower alkyl, lower alkoxy, aryl, halogen and/or by nitro, such as unsubstituted or correspondingly substituted lower alkyl, phenyl-lower alkyl, cycloalkyl or phenyl, for example 2-tri-lower alkylsilylethoxy-carbonyl, such as 2-trimethylsilylethoxycarbonyl or 2-(methyl-di-(n-butyl)-silyl)-ethoxy-carbonyl, or 2-triarylsilylethoxycarbonyl, such as 2-triphenylsilylethoxycarbonyl.

Preferred protected carboxy groups are, for example, tert.-lower alkoxycarbonyl, such as tert.-butoxycarbonyl, and benzyloxycarbonyl that is unsubstituted or substituted, for example, as mentioned above, such as 4-methoxy- or 4-nitrobenzyloxycarbonyl, or diphenylmethoxycarbonyl, and also 2-(trimethylsilyl)-ethoxycarbonyl.

A protected amino group can be, for example, in the form of a readily cleavable acyl-amino, arylmethylamino, etherified mercaptoamino, 2-acyllower alk-1-enylamino, silylamino or stannylamino group or in the form of an azido group.

In a corresponding acylamino group, acyl is, for example, the corresponding radical of an organic carboxylic acid having, for example, up to 18 carbon atoms, especially an alkanecarboxylic acid that is unsubstituted or substituted, for example, by halogen or aryl, or of benzoic acid that is unsubstituted or substituted, for example, by halogen, for example chlorine, lower alkoxy, for example methoxy, nitro, and/or by phenyl, or of a carbonic acid semi-ester. Such acyl groups are, for example, lower alkanoyl, such as formyl, acetyl or propionyl, halo-lower alkanoyl, such as 2-haloacetyl, especially 2-chloro-, 2-bromo-2-iodo-, 2,2,2-trifluoro- or 2,2,2-trichloroacetyl, benzoyl that is unsubstituted or substituted, for example, by halogen, lower alkoxy, nitro and/or by phenyl, for example benzoyl, 4-chlorobenzoyl, 4-methoxybenzoyl or 4-nitrobenzoyl, or lower alkoxycarbonyl that is branched at the 1-position of the lower alkyl radical or suitably substituted at the 1-or 2-position, especially tert.-lower alkoxycarbonyl, for example tert.-butoxycarbonyl, lower alkenyloxycarbonyl, for example allyloxycarbonyl, α-aryl-lower alkoxycarbonyl having one or two aryl radicals that are preferably phenyl that is unsubstituted or substituted, for example, by lower alkyl, especially tert.-lower alkyl, such as tert.-butyl, lower alkoxy, such as methoxy, hydroxy, halogen, for example chlorine, nitro and/or by phenyl, such as unsubstituted or substituted benzyloxycarbonyl, for example 4-nitrobenzyloxycarbonyl, biphenylyl-lower alkoxycarbonyl in which biphenylyl substitutes the α-position, for example 2-(p-biphenylyl)-2-propoxycarbonyl), or substituted diphenylmethoxycarbonyl, for example benzhydryloxycarbonyl or di-(4-methoxyphenyl)-methoxycarbonyl, 2,2-diarylethoxycarbonyl in which aryl is phenyl that is unsubstituted or substituted, for example, by nitro, such as 4-nitrophenyl, such as 2,2-di-(4-nitrophenyl)-ethoxycarbonyl, wherein the two aryl, for example phenyl, radicals may also be bonded to one another, for example 9-fluorenyl-methoxycarbonyl, aroyl-methoxycarbonyl in which the aroyl group is preferably benzoyl that is unsubstituted or substituted, for example, by halogen, such as bromine, for example phenacyloxycarbonyl, 2-halo-lower alkoxycarbonyl, for example 2,2,2-trichloroethoxycarbonyl, 2-bromo-ethoxycarbonyl or 2-iodoethoxycarbonyl, optionally substituted 2-phenylsulfonyl-ethoxy-carbonyl, such as 2-(4-methylsulfonylphenylsulfonyl)-ethoxycarbonyl, or 2-(tri-substituted silyl)-ethoxycarbonyl in which each of the substituents, independently of the others, is an aliphatic, araliphatic, cycloaliphatic or aromatic hydrocarbon radical that has up to 15

carbon atoms and is unsubstituted or substituted, for example, by lower alkyl, lower alkoxy, aryl, halogen or by nitro, such as corresponding unsubstituted or substituted lower alkyl, phenyl-lower alkyl, cycloalkyl or phenyl, for example 2-tri-lower alkylsilylethoxycarbonyl, such as 2-trimethylsilylethoxycarbonyl or 2-(di-n-butyl-methyl-silyl)-ethoxycarbonyl, or 2-triarylsilylethoxycarbonyl, such as 2-triphenylsilylethoxycarbonyl.

Further acyl radicals suitable as amino-protecting groups are also corresponding radicals of organic phosphoric, phosphonic or phosphinic acids, such as di-lower alkylphosphoryl, for example dimethylphosphoryl, diethylphosphoryl, di-n-propylphosphoryl or diisopropylphosphoryl, dicycloalkylphosphoryl, for example dicyclohexylphosphoryl, unsubstituted or substituted diphenylphosphoryl, for example diphenylphosphoryl, di-(phenyl-lower alkyl)-phosphoryl that is unsubstituted or substituted, for example, by nitro, for example dibenzylphosphoryl or di-(4-nitrobenzyl)-phosphoryl, unsubstituted or substituted phenoxyphenylphosphonyl, for example phenoxyphenylphosphonyl, di-lower alkylphosphinyl, for example diethylphosphinyl, or unsubstituted or substituted diphenylphosphinyl, for example diphenylphosphinyl.

In an arylmethylamino group, which may be a mono-, di- or especially tri-arylmethylamino group, the aryl radicals are especially unsubstituted or substituted phenyl radicals. Such groups are, for example, benzylamino, diphenylmethylamino and especially tritylamino.

An etherified mercapto group in an amino group protected by such a radical is especially arylthio or aryl-lower alkylthio in which aryl is especially phenyl that is unsubstituted or substituted, for example, by lower alkyl, such as methyl or tert.-butyl, lower alkoxy, such as methoxy, halogen, such as chlorine, and/or by nitro. A corresponding amino-protecting group is, for example, 4-nitrophenylthio.

In a 2-acyl-lower alk-1-en-1-yl radical that may be used as an aminoprotecting group, acyl is, for example, the corresponding radical of a lower alkanecarboxylic acid, of a benzoic acid that is unsubstituted or substituted, for example, by lower alkyl, such as methyl or tert.-butyl, lower alkoxy, such as methoxy, halogen, such as chlorine, and/or by nitro, or especially of a carbonic acid semi-ester, such as a carbonic acid lower alkyl semi-ester. Corresponding protecting groups are especially 1-lower alkanoylprop-1-en-2-yl, for example 1-acetylprop-1-en-2-yl, or 1-lower alkoxycarbonylprop-1-en-2-yl, for example 1-ethoxycarbonyl-prop-1-en-2-yl.

An amino group can also be protected in protonated form; as corresponding anions there come into consideration especially those of strong inorganic acids, such as hydrohalic acids, for example the chloride or bromide anion, or organic sulfonic acids, such as p-toluenesulfonic acid.

Preferred amino-protecting groups are acyl radicals of carbonic acid semi-esters, especially tert.-butoxycarbonyl, allyloxycarbonyl, or benzyloxycarbonyl that is unsubstituted or substituted, for example as indicated, for example 4-nitrobenzyloxycarbonyl, or diphenylmethoxycarbonyl, 9-fluorenyl-methoxycarbonyl, or 2-halo-lower alkoxycarbonyl, such as 2,2,2-trichloroethoxycarbonyl, and also trityl or formyl.

Unsubstituted carbamoyl groups are protected, for example, in the form of N-(9-xanthenyl) derivatives or in the form of N-(mono-, di- or tri-arylmethyl) derivatives, in which aryl is especially phenyl that is unsubstituted or contains up to 5 identical or different substituents, preferably lower alkyl, such as methyl, or lower alkoxy, such as methoxy. The following may be mentioned as examples of such arylmethyl protecting groups: 4-methoxybenzyl, 2,4,6-trimethoxybenzyl, diphenylmethyl, di-(4-methoxyphenyl)-methyl, di-(4-methylphenyl)-methyl and (4-methylphenyl)-([polymeric carrier]-phenyl)-methyl. A preferred carbamoyl-protecting group is trityl.

Guanidino groups may be protected, for example, by protonation, or by nitro or by means of suitably substituted sulfonyl groups, such as arylsulfonyl in which aryl is phenyl that is unsubstituted or contains, for example, lower alkyl, such as methyl, or benzoheterocyclyl, such as chromanyl, that is unsubstituted or substituted, for example, by lower alkyl, such as methyl, and bonded by way of an aromatic carbon atom, such as 4-methoxy-2,3,5(tri-methyl)-phenylsulfonyl or 2,2,5,7,8-pentamethyl-6-chromanylsulfonyl.

In this application there is to be understood by a protecting group, especially a carboxy-protecting group, also a polymeric carrier that is bonded to the functional group to be protected, especially to a carboxy group, which carrier is suitable especially for the so-called Merrifield peptide synthesis and can be readily removed. Such a polymeric carrier is, for example, preferably a polystyrene resin weakly crosslinked by copolymerisation with divinylbenzene, which resin carries bridging members suitable for the reversible bonding of amino acid and peptide residues. Especially in connection with the above-mentioned weakly crosslinked polystyrene resin, these bridging members are

especially methylene groups that are unsubstituted or substituted and that are bonded directly to aromatic radicals of the polystyrene resin. Substituents of the methylene groups are bonded to the methylene groups preferably by ether or ester groupings and contain suitable functional groupings that together with functional groups, especially carboxy groups, of the amino acid or peptide fragment, can form protected groups, especially corresponding carboxy groups, such as esterified carboxy groups. Such bridging members are, for example, the divalent radicals of 4-methoxybenzyl alcohols optionally containing in the α-position lower alkoxy, such as methoxy, or phenyl that is unsubstituted or substituted, for example in the o- and/or p-position, for example by lower alkoxy, such as methoxy, in which 4-methoxybenzyl alcohols the carbon atom of the 4-methoxy group is bonded directly to a phenyl radical of the polystyrene resin, and the benzylic hydroxy group esterifies the carboxy function of the amino acid or of the peptide fragment.

The reaction to form the amide bond can be carried out in a manner known per se, the reaction conditions depending especially on whether and how the carboxy group that participates in the reaction has been activated, customarily in the presence of a suitable solvent or diluent or a mixture thereof, and, if necessary, in the presence of a condensing agent which, for example, if the carboxy group that participates in the reaction is present in the form of an anhydride, may also be a suitable acid-binding agent, with cooling or heating, for example in a temperature range of from approximately -30°C to approximately +150°C, especially from +10°C to +70°C, preferably from room temperature (approximately +20°C) to +50°C, in a closed reaction vessel and/or in the atmosphere of an inert gas, for example nitrogen.

Customary condensing agents are, for example, carbodiimides, for example N,N'-diethyl-, N,N'-diisopropyl-, N,N'-dicyclohexyl- or N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide, suitable carbonyl compounds, for example carbonyldiimidazole, or 1,2-oxazolium compounds, for example 2-ethyl-5-phenyl-1,2-oxazolium-3'-sulfonate and 2-tert.-butyl-5-methylisoxazolium perchlorate, a suitable acylamino compound, for example 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, furthermore a uronium compound, for example 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), or a phosphonium compound, for example benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) or benzotriazole-1-yl-oxy-pyrrolidino-phosphonium hexafluorophosphate (PyBOP). Customary acid-binding agents are, for example, alkali metal carbonates or bicarbonates, for example sodium or potassium carbonate or bicarbonate (customarily together with a sulfate), or organic bases, such as customarily

sterically hindered tri-lower alkylamines, for example N,N-diisopropyl-N-ethylamine.

The above-mentioned Merrifield peptide synthesis is suitable especially for a semi-automatic or fully automatic synthesis of compounds of formula (2a), wherein amino acids and/or peptide fragments and/or other non-peptidic moieties in which functional groups not participating in the reaction are usually in protected form are linked to one another by way of amide groupings without isolation of the peptide fragments formed. One of the functional groups, normally the terminal carboxy group present in the end peptide, is optionally bonded to a suitable polymeric carrier by a bridging member, as described. In principle this process variant is carried out analogously to the customary synthesis of peptides, care being taken that, in the already synthesized (peptide) fragment that contains the polymeric carrier moiety, the freeing, from the protected group, of the functional group that participates in the reaction, usually the terminal amino group, is in each case carried out under conditions in which the protecting groups of the functional groups not participating in the reaction are retained.

The removal of carboxy-, amino-, hydroxy-, carboxylic acid amide-, carbamoyl- and/or guanidino-protecting groups is carried out in a manner known per se, for example by means of β-elimination, solvolysis, especially hydrolysis (under acid or basic conditions), alcoholysis, acidolysis or treatment with a base, or by means of reduction, especially hydrogenolysis or chemical reduction, optionally in stages or simultaneously, it also being possible to use enzymatic methods.

Thus, tert.-lower alkoxycarbonyl, or lower alkoxycarbonyl substituted in the 2-position by an organic silyl group or in the 1-position by lower alkoxy or lower alkylthio, or unsubstituted or substituted diphenylmethoxycarbonyl, can be converted into free carboxy by acidolysis, for example by treatment with a suitable acid, such as a lower alkane-carboxylic acid which may contain halogen, for example formic acid or trifluoroacetic acid, with or without the addition of a nucleophilic compound, such as phenol or anisole. Unsubstituted or substituted benzyloxycarbonyl can be freed, for example, by hydrogenolysis, that is to say by treatment with hydrogen in the presence of a metallic hydrogenation catalyst, such as a palladium catalyst. In addition, suitably substituted benzyloxycarbonyl, such as 4-nitrobenzyloxycarbonyl, can be converted into free carboxy also by chemical reduction, for example by treatment with an alkali metal dithionite, for example sodium dithionite, or with a reducing metal, for example zinc, or a reducing metal salt, such as a chromium(II) salt, for example chromium(II) chloride, usually in the

presence of a hydrogen donor that, together with the metal, is capable of producing nascent hydrogen, such as an acid, especially a suitable carboxylic acid, such as a lower alkanecarboxylic acid that is unsubstituted or substituted, for example, by hydroxy, for example acetic acid, formic acid, glycolic acid, diphenylglycolic acid, lactic acid, mandelic acid, 4-chloromandelic acid or tartaric acid, or an alcohol or thiol, water preferably being added. 2,2-Diarylethoxycarbonyl or 2-(9-fluorenyl)-ethoxycarbonyl groups can be cleaved under mild basic conditions, for example by treatment with piperidine. By treatment with a reducing metal or metal salt, as described above, it is also possible to convert 2-halo-lower alkoxycarbonyl (optionally after conversion of a 2-bromo-lower alkoxycarbonyl group into a corresponding 2-iodo-lower alkoxycarbonyl group) or aroylmethoxycarbonyl into free carboxy, it being possible to cleave aroylmethoxycarbonyl also by treatment with a nucleophilic, preferably salt-forming, reagent, such as sodium thiophenolate or sodium iodide. Substituted 2-silylethoxycarbonyl can also be converted into free carboxy by treatment with a salt of hydrofluoric acid yielding the fluoride anion, such as an alkali metal fluoride, for example sodium or potassium fluoride, in the presence of a macrocyclic polyether ("crown ether"), or with a fluoride or an organic quaternary base, such as tetra-lower alkylammonium fluoride or tri-lower alkylarylammonium fluoride, for example tetraethylammonium fluoride or tetrabutylammonium fluoride, in the presence of an aprotic polar solvent, such as dimethyl sulfoxide or N,N-dimethylacetamide.

A protected amino group is freed in a manner known per se and, depending on the nature of the protecting groups, by various methods, but preferably by solvolysis or reduction. 2-Halo-lower alkoxycarbonylamino (optionally after conversion of a 2-bromo-lower alkoxycarbonylamino group into a 2-iodo-lower alkoxycarbonylamino group), aroylmethoxycarbonylamino or 4-nitrobenzyloxycarbonylamino can be cleaved, for example, by treatment with a suitable chemical reducing agent, such as zinc in the presence of a suitable carboxylic acid, such as aqueous acetic acid. Aroylmethoxycarbonylamino can also be cleaved by treatment with a nucleophilic, preferably salt-forming, reagent, such as sodium thiophenolate, and 4-nitrobenzyloxycarbonylamino also by treatment with an alkali metal dithionite, for example sodium dithionite. Unsubstituted or substituted diphenylmethoxycarbonylamino, tert.-lower alkoxycarbonylamino or 2-trisubstituted silylethoxycarbonylamino can be cleaved by treatment with a suitable acid, such as a lower alkanecarboxylic acid that is unsubstituted or substituted, for example, by halogen, such as fluorine, for example formic acid or trifluoroacetic acid, and 2,2-diarylethoxycarbonylamino, such as 2,2-di-(4-nitrophenyl)-ethoxycarbonylamino, 2-(4-methylsulfonyl-

phenylsulfonyl)-ethoxycarbonyl and also 9-fluorenyl-methoxycarbonylamino by treatment with a suitable base, such as an aliphatic, preferably secondary, amine, for example piperidine. The amino group can be freed from unsubstituted or substituted benzyloxycarbonylamino, for example, by hydrogenolysis, that is to say by treatment with hydrogen in the presence of a suitable hydrogenation catalyst, such as a palladium catalyst, from unsubstituted or substituted triarylmethylamino or formylamino, for example, by treatment with an acid, such as a mineral acid, for example hydrochloric acid, or an organic acid, for example formic, acetic or trifluoroacetic acid, in the presence or absence of water, and from an organic silylamino group, for example, by hydrolysis or alcoholysis. An amino group protected by 2-haloacetyl, for example 2-chloroacetyl, can be freed, for example, by treatment with thiourea in the presence of a base, or with a thiolate salt, such as an alkali metal thiolate, of thiourea, and by subsequent solvolysis, such as alcoholysis or hydrolysis, of the resulting condensation product. An amino group protected by 2-substituted silylethoxycarbonyl can also be converted into the free amino group by treatment with a salt of hydrofluoric acid yielding fluoride anions, as described above in connection with the freeing of a correspondingly protected carboxy group.

Amino protected in the form of an azido group can be converted into free amino, for example, by reduction, for example by catalytic hydrogenation with hydrogen in the presence of a hydrogenation catalyst, such as platinum oxide, palladium or Raney nickel, or alternatively by treatment with zinc in the presence of an acid, such as acetic acid. The catalytic hydrogenation can preferably be carried out in an inert solvent, such as an alcohol, for example methanol, tetrahydrofurane, or alternatively in water or a mixture of water and an organic solvent, such as an alcohol or dioxan, at approximately from 20°C to 25°C, or alternatively with cooling or heating.

A hydroxy group protected by a suitable acyl group, an organic silyl group or by unsubstituted or substituted 1-phenyl-lower alkyl can be released analogously to a correspondingly protected amino group. A hydroxy group protected by 2,2-dichloroacetyl can be released, for example, by basic hydrolysis, and a hydroxy group etherified by tert.-lower alkyl, for example tert.-butyl, or by a 2-oxa- or 2-thia-aliphatic or -cycloaliphatic hydrocarbon radical can be released by acidolysis, for example by treatment with a mineral acid or a strong carboxylic acid, for example trifluoroacetic acid.

A carboxylic acid amide group protected by 9-xanthenyl can be released, for example, by treatment with hydrogen bromide in glacial acetic acid or with hydrogen fluoride in the

presence of anisole. A carboxylic acid amide group protected by mono-, di- or tri-arylmethyl can be released, for example, by treatment with hydrogen fluoride in the presence of anisole; furthermore, a diphenylmethyl protecting group can be removed, for example, by hydrogenolysis in the presence of a palladium-on-carbon catalyst, and a di-(4-methoxyphenyl)-methyl protecting group or a 2,4,6-trimethoxybenzyl protecting group can be removed, for example, by treatment with trifluoroacetic acid.

Guanidino groups protected by organic sulfonyl groups, such as 4-methylphenylsulfonylguanidino or 2,2,5,7,8-pentamethyl-6-chromanylsulfonylguanidino, can be released, for example, by treatment with a suitable acid, such as trifluoroacetic acid. Guanidino groups protected by nitro can be released, for example, by hydrogenolysis in the presence of palladium-on-carbon catalyst or cationic reduction.

A protected functional group, especially a corresponding carboxy group, in which the protecting group simultaneously acts as a carrier material in the mentioned Merrifield peptide synthesis, can be cleaved in a manner known per se, for example as described above. A correspondingly esterified carboxy group that is bonded to the polymeric carrier material by way of a suitable bridging member is cleaved in accordance with the nature of the bridging member. For example, a carboxy group bonded to the polymeric carrier material by way of an ester grouping having an activated benzylic bridging member, for example a 4-methoxybenzyloxycarbonyl group, in which the carbon atom of the methoxy group is bonded, for example, to a phenyl radical of the polystyrene resin weakly crosslinked with divinylbenzene, can be released analogously to the above-mentioned unsubstituted or substituted benzyloxycarbonyl groups, for example by treatment with a suitable acid, such as trifluoroacetic acid.

If desired and if several protected functional groups are present, the protecting groups can be selected in order to facilitate the removal of more than one of these protecting groups at the same time, for example by acidolysis, such as by treatment with trifluoroacetic acid or formic acid, or by reduction, such as by treatment with zinc and acetic acid, or with hydrogen and a hydrogenation catalyst, such as a palladium-on-carbon catalyst.

The cyclization is normally performed after the complete synthesis of the linear precursor, e.g. by formation of a peptide bond, as described above, between the corresponding amino acids and subsequent removal of the remaining protecting groups. If the cyclization is based on a disulfide or sulfide bond, the protecting groups are removed usually before the

oxidative ring formation, e.g. via oxygen, normal air, iodine, disodiumdisulfide, 1,2-diiodo-ethane or other oxidizing agents. The formed disulfide bond can be modified further, e.g. by desulfurization to a thioether radical and oxidation.

The invention relates also to a pharmaceutical composition which consists of one or more of the compounds of formula (1) in a pharmaceutically effective amount, or which comprises said compounds together with conventional auxiliaries, typically carriers and diluents.

The pharmaceutical compositions of this invention are those for enteral, oral, rectal and parenteral administration, as for subcutaneous, intravenous or intraperitoneal administration to warm blooded animals, said compositions containing the pharmacological active compound alone or together with a pharmacologically acceptable carrier. The daily dosage, the carrier, the frequency and route of administration will depend on the age and individual condition of the patient as well as on the mode of administration and the individual activity of the selected compound. A bone resorption inhibitory effective amount of a compound of the formula (1) for a patient with of about 70 kg weight may comprise from 1 µg to 1 g or more. The dosage units may comprise about 10 µg to 10 mg, preferably 10 µg to 500 µg and more preferably 10 µg to 100 µg of the active compound.

The novel pharmaceutical compositions contain from about 10 to 80 %, preferably from 20 to about 60 %, of the active compound. Pharmaceutical compositions for enteral or parenteral administration are typically those in dosage unit forms such as dragées, tablets, capsules or suppositories, and also ampoules. These dosage forms are prepared in a manner known per se, typically by conventional mixing, granulating, confectioning, dissolving or lyophilising methods.

Suitable carriers are especially fillers such as sugars, conveniently lactose, saccharose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, typically tricalcium phosphate or calcium hydrogen phosphate, and also binders such as starch pastes, conveniently using maize, corn, rice or potato starch, gelatin, tragacanth, methyl cellulose and/or polyvinyl pyrrolidone, and/or, if desired, disintegrators such as the above-mentioned starchés, also carboxymethyl starch, crosslinked polyvinylpyrrolidone, agar, alginic acid or a salt thereof such as sodium alginate. Excipients are in particular glidants, flow control agents and lubricants, conveniently silica, talcum, stearic acid or

salts thereof, typically magnesium stearate or calcium stearate, and/or polyethylene glycol. Dragée cores can be provided with suitable non-enteric or enteric coatings, typically using concentrated sugar solutions which may contain gum arabic, talcum, polyvinyl-pyrrolidone, polyethylene glycol and/or titanium dioxide, shellac solutions in suitable organic solvents or mixtures of solvents or, for the preparation of enteric coatings, solutions of suitable cellulose preparations such as acetyl cellulose phthalate or hydroxy-propyl methyl cellulose phthalate. Dyes or pigments can be added to the tablets or dragée coatings, conveniently to identify or indicate different doses of active compound.

Further pharmaceutical compositions for oral administration are dry-filled capsules made of gelatin and also soft-sealed capsules consisting of gelatin and a plasticiser such as glycerol or sorbitol. The dry-filled capsules can contain the active ingredient in the form of granules, conveniently in admixture with fillers such as lactose, binders such as starches, and/or glidants such as talcum or magnesium stearate, and with or without stabilisers. In soft capsules, the active ingredient is preferably dissolved or suspended in a suitable liquid, typically a fatty oil, paraffin oil or a liquid polyethylene glycol, to which a stabiliser can also be added.

Suitable pharmaceutical compositions for rectal administration are typically suppositories, which consist of a combination of the active compound with a suppository base. Examples of suitable suppository bases are natural or synthetic triglycerides, paraffin hydrocarbons, polyethylene glycols and higher alkanols. It is also possible to use gelatin capsules for rectal administration that contain a combination of the active compound with a base substance. Suitable base substances are typically liquid triglycerides, polyethgylene glycol or paraffin hydrocarbons.

Compositions for parenteral administration are preferred, conveniently in the form of aqueous solutions of non toxic salts or suspensions of the active compound, for example oily injection suspensions using suitable lipophilic solvents or vehicles such as fatty oils, typically sesame oil, or synthetic fatty acid esters such as ethyl oleate or triglycerides, or aqueous injection suspensions which may contain viscosity increasing substances, conveniently sodium carboxymethyl cellulose, sorbitol and/or dextran, and also with or without stabilisers.

The invention also relates to the use of the compound of formula (1), preferably in the form of pharmaceutical compositions. The dosage of the active compound will depend on

the species of the warm-blooded animal, on the age and individual condition of the patient, and also on the mode of application.

Another embodiment of the invention relates to compound of formula (1) for use in a method for the therapeutic treatment of the human or animal body.

The inventive compounds can be used for the treatment of excess bone resorption e.g. osteoporosis, Paget's disease of bone, humoral hypercalcemia or malignancy as well as metastic bone diseases.

The invention is illustrated in more detail by the following non-limitative Examples.

Abbreviations:

Ac =	acetyl group
Acm =	acetamido-methyl
Boc =	tert. butyloxycarbonyl-
brine=	saturated NaCl solution in H_2O
Bzl =	benzyl
DCCI =	dicyclohexylcarbodiimide
DCE =	1,2-dichloroethan
DCM =	dichloromethane
DIBAH =	diisobutyl aluminum hydride
DICD =	diisopropylcarbodiimide
DIPE =	diisopropylether
DIPEA =	diisopropylethylamine
DMA =	dimethylacetamid
DMAP =	p-N-dimethylamino-pyridine
DME =	1,2-dimethoxyethane
Dpr =	L-diamino-propionic acid
Fmoc =	9-fluorenyl-methoxycarbonyl
HOBt =	1-hydroxy-benzotriazol
NArg =	glycine derivative N-substituted with an arginine-side chain
Pmc =	2,2,5,7,8-pentamethylchroman-6-sulfonyl-
TBTU =	2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborat (R. Knorr et al., (1989) THL 30, 1927-1930)
TCA =	trichloroacetic acid
TFA =	trifluoroacetic acid
TFE =	trifluoroethanol
THF =	tetrahydrofurane
Tra =	tryptamin
Trt =	trityl
MALDI =	matrix-assisted laser desorption ionization mass spectrometry
Tlc =	Thin-layer chromatography on silica (Merck-Kieselgel 60, F254 plates), Detection: UV-light. CMW = mixture of chloroform, methanol and water (70:30:5 v/v); CM = mixture of chloroform and methanol (85:15 v/v);
Z =	Benzyloxy carbonyl

TAB-ester resin = hydroxy-trialkoxy-benzhydryl-resin
Wang-resin = Fmoc-'C-terminal amino acid'-p-benzyl oxybenzyl ester polystyrene resin

Unless stated otherwise, all reactions are performed at room temperature.

General process for the solid-phase synthesis of linear precursors:

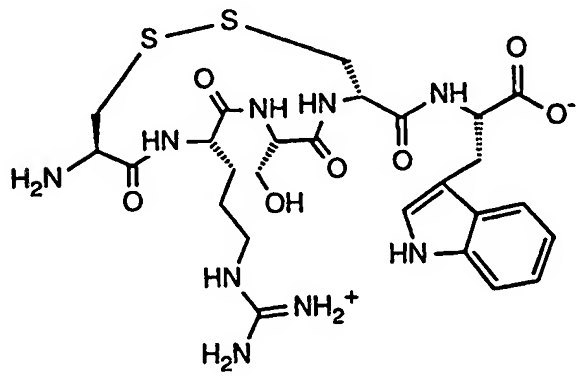
The synthesis starts with a Fmoc-'C-terminal amino acid'-p-benzyl oxybenzyl ester polystyrene resin (1 % crosslinked, 'Wang-resin, Novabiochem, Laeufelfingen, Switzerland). A fully automatic peptide synthesis apparatus is used, that is suitable for alternate removal of the amino-protecting groups, in the present case the Fmoc-group, and coupling of the Fmoc-amino acid derivatives without isolation of the peptide/resin intermediates obtainable at each step. Trifunctional amino acids are introduced as correspondingly protected derivatives of L- or D-configuration: Fmoc-Ser(But), Fmoc-Cys(Trt), Fmoc-Arg(Pmc), Boc-Dpr(Fmoc), Fmoc-Trp(Boc) or Fmoc-Trp.

In a first step the penultimate Fmoc amino acid is coupled with the last amino acid on the resin, and then the other Fmoc amino acids are coupled in steps, in the appropriate sequence. The individual steps are carried out in accordance with the following scheme, approximately 20 ml of the washing liquids being used in each case, with the individual operations, and the reaction mixture being shaken regularly.

For the synthesis of protected linear precursors the hydroxy-trialkoxy-benzhydryl-resin (TAB) (= TAB-ester resin, Hans Rink, (1987) Tetrahedron Lett. 28, 3787-3790), available from NOVABIOCHEM, is used.

For the synthesis of carboxy-terminal peptide amides the amino-trialkoxy-benzhydryl-resin (= TAB-amide resin, Hans Rink, Tetrahedron Lett. (1987), 28, 3787-3790), available from NOVABIOCHEM, is used.

Example 1: Synthesis of a compound of the formula (3)



(3)

Example 1.1: Synthesis of the linear precursor

Starting from 1.12 g of Fmoc-Trp-Wang-resin (about 0.35 mMole, Novabiochem), the following process steps, repeated for each step, are carried out:

- single wash for 0.8 minutes with isopropanol;

- pre-activation for first coupling: 1.4 mMole of the respective appropriately protected Fmoc-amino acid are dissolved in 3.08 ml of 0.5 M HOBt in DMA and 0.77 ml of a 2 M solution of DICD in DMA are added. The reaction mixture is maintained for about 40 minutes, and then used in that form. During this time the following washings and the removal of the Fmoc protecting group is already proceeding;

- eight treatments of the resin starting material, each of 1 minute's duration, with a 20 % solution of piperidine in DMA (removal of the Fmoc protecting group);

- three washes, each of 0.4 minute's duration, with DMA (previously degassed under reduced pressure);

- single wash for 0.8 minutes with isopropanole;

- five washes, each of 0.4 minute's duration, with DMA (degassed);

- addition of the coupling reagent, which has been prepared in the meantime (see above). The reaction mixture is maintained with shaking for about 60 minutes. Twice, 5 minutes and 15 minutes after beginning of the coupling, 120 µl DIPEA (0.7 mMole) are added.

- removal of the coupling mixture after 60 minutes;

- two washes for 0.4 minutes with DMA (degassed);

- single treatment for 5 minutes with approximately 15 ml of a 1:1:8 mixture (v/v/v) of acetic anhydride, pyridine and DMA (for the acetylation of amino groups that are still free in the growing peptide chain);

- four washes, each of 0.4 minute's duration, with DMA (degassed);

- two washes, each of 0.8 minute's duration, with isopropanol.

In this manner there is obtained the following Fmoc-peptide/resin intermediate:

Fmoc-Cys(Trt)-Arg(Pmc)-Ser(But)-D-Cys(Trt)-Trp-resin by sequential coupling of Fmoc-D-Cys(Trt), Fmoc-Ser(But), Fmoc-Arg(Pmc) and Fmoc-Cys(Trt); wherein "resin" denotes the carboxy group-esterifying polystyrene (1 % crosslinked with divinylbenzene)-methoxy-4-phenylmethoxy radical.

After removal of the Fmoc protecting group at the N-terminal cysteine residue, and extensive washing with DMA and isopropanol as described above, the resin is dried in vacuo (1.45 g).

<u>Example 1.2: Removal of the resin and the protecting groups</u>

In order to remove the polymeric carrier, and the acid-labile protecting groups, 0.3 g of the Cys(Trt)-Arg(Pmc)-Ser(But)-D-Cys(Trt)-Trp-resin (approximately 63 μmol) are shaken twice for 5 minutes with 4 ml of a 85:15 mixture (v/v) of TFA (95 %) and ethanedithiole and then filtered. The filtration residue is then washed three times with 4 ml each of DCE and three times with 4 ml each of TFE. The combined filtrates and washing liquids are concentrated under reduced pressure to a volume of about 2 ml, and the crude peptide is precipitated by the addition of 15 ml of a 1:1 mixture (v/v) of DIPE and petroleum ether (low-boiling). The precipitate is isolated by filtration, washed with 5 ml of the precipitation mixture and dried under reduced pressure.

For completely removing the protecting groups, the solid (64 mg) is dissolved in 4 ml of the above mentioned cleavage mixture and kept for two hours, precipitated and dried

under reduced pressure.

Example 1.3: Cyclization of the linear peptide Cys-Arg-Ser-D-Cys-Trp

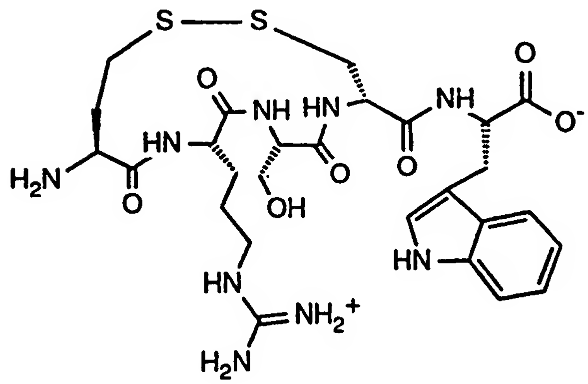
72.5 mg of the linear peptide is dissolved in 1100 ml water and the pH adjusted to 8 by addition of 25 µl ammonia (10 % in water). The cyclization by disulfide formation is performed by bubbling air through the solution for 4 hours. After acidification with 5 ml AcOH, the solution is concentrated under reduced pressure to about 10 ml and lyophilized.

For purification, 67 mg of the crude peptide so obtained are dissolved in a mixture of 4.5 ml CH_3CN/water (1:9 v/v) and 0.1 ml AcOH and subjected to high pressure liquid chromatography (HPLC) under the following conditions: the column, measuring 20x250 mm, Nucleosil $7C_{18}$ (10 nm) manufactured by Machery-Nagel, Dueren, Germany; 0.1 % TFA is used as eluent (A) and a 0.1 % solution of TFA in CH_3CN is used as eluent (B). The linear gradient is 10 % B to 50 % B in 30 minutes, the throughput speed 18 ml/min and the detection is at 215 nm wavelength. The main fraction, with a retention time of approximately 11 minutes, is collected, concentrated under reduced pressure and filtrated through an ion-exchange column (15 ml), AG1-X8 (Bio-Rad), preloaded with AcOH, for exchanging TFA with AcOH. The column is extensively washed with water and the combined fractions (40 ml) are lyophilized. The compound with the formula (3) is obtained as a colorless, fluffy powder.

Analytics:
HPLC: column measuring 4.6x250 mm, Nucleosil $7C_{18}$ (10 nm) manufactured by Machery-Nagel, Dueren, Germany; 0.1 % TFA is used as eluant (A) and a 0.1 % solution of TFA in CH_3CN is used as eluant (B). The linear gradient is 10 % B to 90 % B in 30 minutes, 1 ml/min, detection at 215 nm wavelength. Retention time of the single peak is 13.1 min.
MALDI, negative mode: 650.7 (calculated mass: 650.8).

^{1}H-NMR (TOCSY and ROESY experiments): in a mixture of water/D_2O (95:5 v/v) at 25°C, chemical shifts (ppm) relative to DSS: Cys(1), αH 4.11, ßH 3.31, 2.96; Arg, NH 8.94, αH 4.37, ßH 1.84, 1,81, γH 1.66, 1.60, δH 3.20, 3.20, guanido-NH 7.16, 6.61; Ser, NH 8.56, αH 4.26, βH 3.78, 3.76; Cys(4), NH 7.71, αH 4.53, βH 3.06, 2.89, Trp, NH 7.85, αH 4.59, βH 3.38, 3.10, 2H 7.18, 4H 7.68, 5H 7.14, 6H 7.22, 7H 7.47, NH 10.00.

Example 2: Synthesis of a compound of the formula (4)



(4)

Example 2.1: Linear peptide precursor

The linear intermediate of the compound of the formula (4) is prepared in an analogues manner as described in example 1.1 and 1.2, but L-homo-cysteine is incorporated in place of L-cysteine (Fmoc-homo-Cys(Trt) from Bachem).

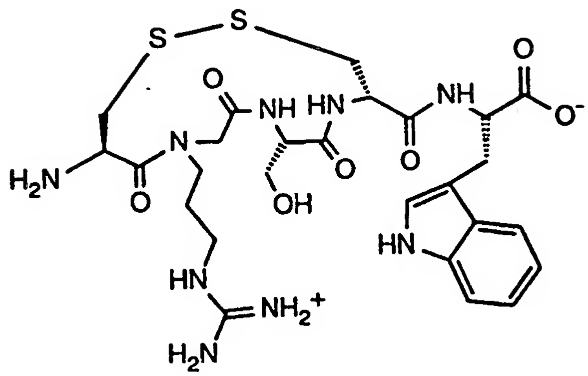
Example 2.2: Oxidation of Homo-Cys-Arg-Ser-D-Cys-Trp

40 mg of the linear peptide is dissolved in 600 ml water and a solution of 900 µl 0.1 M iodine in 95 % AcOH is added during 15 minutes. After 1 hour, 10 mg ascorbic acid is added for reducing an excess of iodine, resulting in a colorless solution which is concentrated to 30 ml under reduced pressure at 30°C.

This solution is lyophilized and the resulting white powder (ca. 60 mg) is subjected to HPLC purification as in example 1.3. The compound of the formula (4) is obtained as a fluffy white powder.

Analytics:
analytical HPLC as described, retention time of the single peak is 13.7 minutes.
MALDI, positive mode: 666.7 (calculated mass: 666.8).

Example 3: Synthesis of a compound of the formula (5)



(5)

Example 3.1: Synthesis of Fmoc-NArg(Pmc)

Example 3.1.1: Synthesis of N-Benzyl-N-3-phthalimidopropylglycinebenzylester

16 g (62.6 mmol) of N-benzylglycinebenzylester is dissolved in 160 ml of dry DMF. To this solution are added 21 g (78.2 mmol) of 3-bromopropylphthalimid and 13.4 ml (78.2 mmol) of diisopropylethylamine. The reaction mixture is stirred for 16 hours at 60°C bath temperature under the exclusion of moisture. DMF is evaporated under reduced pressure and the oily residue is chromatographed on 300 g silica gel (70-230 mesh) with petrol ether/ethyl acetate 95:5 to 85:15 to give the N-Benzyl-N-3-phthalimidopropyl-glycinebenzylester.

TLC: $R_f = 0.32$ (petrol ether/ethyl acetate 4:1)
^{1}H-NMR ($CDCl_3$;300 MHz): 7.85 (2H); 7.71 (2H) phthalimido; 7.38-7.24(10H) benzyl; 5.13 (2H) CH_2-glycine; 3.78 (2H) OCH_2; 3.75 (2H); 3.39 (2H); 2.77 (2H) 3xN-CH_2; 1.88 (2H) CH_2-propyl;

Example 3.1.2: Synthesis of N-3-Aminopropyl-N-benzylglycine

17.4 g (39.3 mmol) of N-benzyl-N-3-phthalimidopropylglycinebenzylester is suspended in 100 ml of 6N HCl. The suspension is stirred under reflux for 6 hours. On cooling phthalic acid separates from the reaction mixture. The phthalic acid is filtered off and washed with 4N HCl. The filtrate is evaporated to a small volume (about 20 ml) and

several times coevaporated with methanol. The oily residue is dissolved with 60 ml of ethanol and the excess HCl is removed by the addition of propylene oxide. N-3-aminopropyl-N-benzylglycine is obtained by the addition of acetone. The colorless solid is filtered and washed with acetone and diethyl ether and dried in a desiccator over potassium hydroxide.

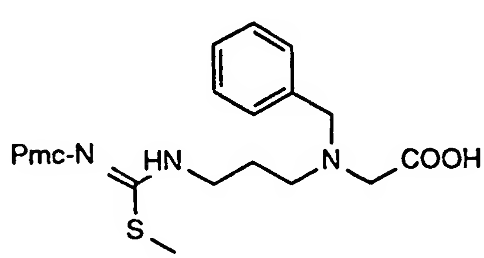
TLC: $R_f = 0.44$ ($CHCl_3$/MeOH/AcOH/H_2O)
MALDI: 223.9 (MH+) found for $C_{12}H_{18}N_2O_2$ (222.29)

Example 3.1.3: Synthesis of Dimethyl(2,2,5,7,8-pentamethylchroman-6-sulfonylimino) dithio carbonate (Pmc-NC(SCH_3)$_2$)

943 mg (3.33 mmol) 2,2,5,7,8-pentamethylchroman-6-sulfonamide is dissolved in 3 ml dry DMF. 200 µl 20N sodium hydroxide solution (4 mmol) and 139.3 mg (1.83 mmol) CS_2 are added under the exclusion of oxygen. Additional 200 µl 20N sodium hydroxide solution and 139.3 mg CS_2 are added in two portions after 10 and 20 minutes respectively. To the suspension are added 415.6 µl (6.66 mmol) methyliodide and the reaction mixture is stirred for two hours. The obtained solution is diluted with 30 ml of ethyl acetate and extracted with 30 ml of water. The organic layer is dried with sodium sulfate and evaporated to dryness. The crude product is purified by chromatography on 20 g silica gel (70-230 mesh) with petrol ether/ethyl acetate 95:5 to 85:15.

TLC: $R_f = 0.44$ (petrol ether/ethyl acetate 4:1)
^{1}H-NMR ($CDCl_3$;300 MHz): 2.66 (2H); 1.84 (2H) 2xCH_2 chroman; 2.58 (3H); 2.55 (3H); 2.15 (3H) 3xCH_3 chroman; 2.52 (6H) 2xSCH_3; 1.34 (6H) 2xCH_3 chroman;

Example 3.1.4: Synthesis of a compound of the formula (5.1)



(5.1)

565 mg (1.914 mmol) N-3-aminopropyl-N-benzylglycine (example 3.1.2) is suspended in 22.5 ml of dry ethanol. 675 mg (1.74 mmol) Pmc-$NC(SCH_3)_2$ (example 3.1.3) and 1.84 ml (10.77 mmol) of diisopropylethylamine are added. The reaction mixture is stirred over night at 60°C bath temperature. The ethanol is evaporated and the crude residue is chromatographed on 20 g silica gel (70-230 mesh) with dichloromethane/methanol 100:1 to 100:10 to give S-Methyl-N-(2,2,5,7,8-pentamethylchroman-6-sulfonyl)-N'-(3-N -benzylglycine)propylisothiourea (5.1).

TLC: $R_f = 0.26$ (dichloromethane / methanol 9:1)
MALDI: 563.07 (MH+) found for $C_{28}H_{39}N_3S_2O_5$ (561.76)
^{1}H-NMR ($CDCl_3$; 300 MHz): 8.22 (1H) NH; 7.35 (5H); 3.92 (2H); 3.45 (2H); 3.37 (2H); 2.84 (2H) 4xNCH_2; 2.65 (2H); 1.88 (2H) 2xCH_2 chroman; 2.58 (3H); 2.55 (3H); 2.17 (3H) 3xCH_3 chroman; 2.38 (3H) SCH_3; 1.91 (2H) CH_2-propyl; 1.33 (6H) 2xCH_3 chroman;

Example 3.1.5: Substitution of the -SCH_3-group with -NH_2

715 mg (1.27 mmol) S-methyl-N-(2,2,5,7,8-pentamethylchroman-6-sulfonyl)-N'-(3-N-benzylglycinyl)propylisothiourea (17) are dissolved in 24 ml of acetonitrile saturated with anhydrous ammonia. The solution is cooled with an ice bath and the solution of 208.5 mg (1.23 mmol) silver nitrate in 6 ml acetonitrile is added dropwise and the reaction mixture is stirred at room temperature over night. A precipitate ($AgSCH_3$) is formed and filtered off. The acetonitrile is evaporated under reduced pressure and the residue is dissolved in 25 ml of ethyl acetate and washed with 0.1N HCl and brine. The organic layer is dried over sodium sulfate and again evaporated to dryness. The crude product is chromatographed on 20 g silica gel (70-230 mesh) with dichloromethane/methanol 100:2 to 100:20 to give N-(2,2,5,7,8-pentamethylchroman-6-sulfonyl)-N'-(3-N-benzyl-glycinyl)propylguanidine

TLC: $R_f = 0.12$ (dichloromethane / methanol 9:1)
MALDI: 532.24 (MH+) found for $C_{27}H_{38}N_3SO_5$ (530.68)
^{1}H-NMR (DMSO-d^6/D_2O; 300 MHz): 7.23 (5H); 3.65 (2H); 3.12 (2H); 3.05 (2H) 3xNCH_2; 2.58 (2H); 1.75 (2H) 2xCH_2 chroman; 2.47 (6H); 2.01 (3H) 3xCH_3 chroman; 1.54 (2H) CH_2-propyl; 1.24 (6H) 2xCH_3 chroman;

Example 3.1.6: Removal of the benzyl group

630 mg (1.19 mmol) N-(2,2,5,7,8-pentamethylchroman-6-sulfonyl)-N'-(3-N-benzyl-glycinyl)propylguanidine (example 3.1.5) are dissolved in 20 ml dry methanol and hydrogenated with 130 mg of 10 % Pd/C. After 17 hours the theoretical amount of hydrogen is consumed and the reaction is stopped. The catalyst is filtered off and the filtrate is evaporated to dryness. The obtained foam (N-(2,2,5,7,8-pentamethylchroman-6-sulfonyl)-N'-(3-glycinyl)propylguanidine) is dried under high vacuum and is used for the next step without further purification.

TLC: $R_f = 0.38$ (MeOH with 1 % AcOH)
MALDI: 441.9 (MH+) found for $C_{20}H_{32}N_4SO_5$ (440.56)

Example 3.1.7: Introduction of the Fmoc protecting group

520 mg (1.18 mmol) N-(2,2,5,7,8-pentamethylchroman-6-sulfonyl)-N'-(3-glycinyl)propyl-guanidine are suspended in 2.83 ml 9 % sodium carbonate solution and 2.83 ml of dimethylformamide. The reaction mixture is cooled in an ice bath and the solution of 335 mg (0.99 mmol) succinimidyl-9-fluorenylmethyl carbonate in 2.2 ml of DMF is added in one portion. The mixture is sonicated to form a well divided suspension. After stirring for 45 minutes at room temperature the reaction mixture is diluted with 30 ml of water and extracted with ethyl acetate (2x 75 ml). The remaining aqueous phase is acidified to pH 2 with 4N HCl and again extracted with ethyl acetate (3x 30 ml). The combined organic phases are dried over sodium sulfate and evaporated to dryness. The purified product is obtained by chromatography on 20 g silica gel (70 - 230 mesh) with dichloromethane/methanol 100:1 to 100:10 to give N-(2,2,5,7,8-pentamethylchroman-6-sulfonyl)-N'-(3-N-9-fluorenylmethoxycarbonylglycinyl)propylguanidine (Fmoc-NArg(Pmc)). The preparation of Fmoc-NArg(Pmc) has been described by Simon et al., Proc. Natl. Acad. Sci USA (1992), 89, 9367-9371.

TLC: $R_f = 0.52$ (dichloromethane/methanol 9:1)
MALDI: 662.86 (MH+) found for $C_{35}H_{42}N_4SO_7$ (662.80).

Example 3.2: Linear peptide precursor

The linear intermediate of the compound of the formula (5) is prepared in an analogues manner as described in example 1.1 and 1.2, but NArg is incorporated in place of Arg.

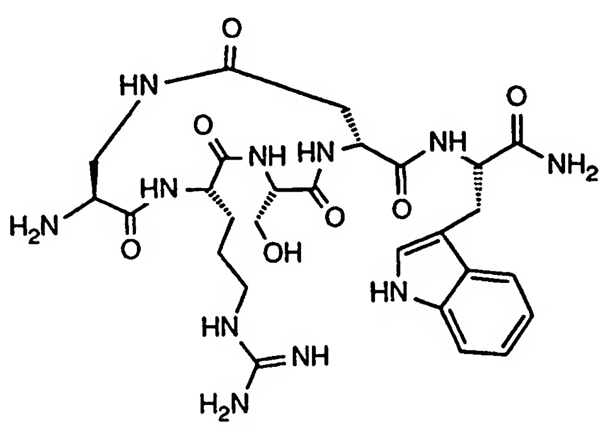
Example 3.3: Oxidation of Cys-NArg-Ser-D-Cys-Trp

70 mg of the linear precursor are dissolved in 1000 ml water and the pH of the solution is adjusted to 8.0 by addition of concentrated ammonia (ca. 0.5 ml). Air is bubbled through the solution for 22 hours. After addition of 15 ml AcOH the solution is concentrated and lyophilized.

The crude material is purified with HPLC and converted to the acetate as described in example 1.3. The compound of the formula (5) is obtained as a fluffy white powder.

Analytics:
homogeneous in the standard Tlc, analytical HPLC as described, retention time of the single peak is 13.1 minutes.
MALDI, positive mode: 652.8 (calculated mass: 653.4).

Example 4: Synthesis of a compound of the formula (6)



(6)

Example 4.1: Synthesis of diamiopropionic acid(Fmoc) (Dpr(Fmoc))

6.04 g L-diamino-propionic acid (Fluka) (43.0 mmol) and 68.8 g basic copper-carbonate (68.8 mmol) are suspended in 144 ml water and refluxed for 1 hour. Insoluble components are removed by filtration, the residue washed with 10 ml water (70°C). 50 ml DMA is added to the resulting deep-blue copper-diamino-propionate solution and the pH is adjusted to ca. 9 with triethylamine (ca. 1 ml). 15.23 g Fluorenyl-methoxy-carbonyl-N-hydroxy-succinimidate (Novabiochem) is dissolved in 250 ml DMA and this solution is added to the copper-complex and slow stirring during 0.5 hour. The pH is kept at 8-9 by addition of triethylamine (total 6 ml). The solution is kept for 1.5 hour. The mixture is concentrated to 40 ml under reduced pressure at 30-40°C and under stirring slowly added to 950 ml water (0-5°C). After stirring for 1 hour, the precipitate is filtered, washed with water (180 ml) and dried over P_2O_5: 10.3 g. After treatment with 50 ml DIPE, the solid is filtrated and dried under reduced pressure: 8.9 g of blue-colored of Fmoc-Dpr-copper-complex is obtained.

In order to remove the copper, the complex (12.46 mmol) is dissolved in 1100 ml of a mixture of ethanol and water (1:1 v/v), and 4.0 g ethylene-diamino-tetra-acetic acid (EDTA) (13.71 mmol) are added. The mixture is refluxed for 40 minutes and solid components are removed by filtration. The solution is cooled to 0-5°C and after 15 hours, the precipitate is isolated by filtration and dried under reduced pressure. After treatment with 40 ml isopropanole at 50°C, the suspension is maintained at 0-5°C for 1 hour and filtrated. The solid is dried under reduced pressure: 2.76 g white powder.

Tlc CMW: main spot with one minor by-component R_f ca. 0.25. Analytical HPLC as described, retention time 18.8 min.

Example 4.2: Synthesis of Boc-L-diamino-propionic acid(Fmoc)

0.119 g Dpr(Fmoc) (365 (µmol) is suspended in 1.3 ml DMA (dest.), 50.8 µl (365 µmol) triethylamine and 0.087 g Boc-anhydride (401 µmol) (Fluka) is added and the mixture is stirred for 1.5 hour. 1 ml water is added and, after cooling to 0-5°C, the mixture is acidified (ca. pH 4) with 120 µl 2N HCl. The white emulsion is extracted with 70 ml ethylacetate and the organic phase is washed three times with water (20 ml each). After drying over sodium sulfate, the solvent is evaporated under reduced pressure, the residue

is dissolved in 1.3 ml tert. butanol and lyophilized. The compound is obtained as a white powder.

Tlc CMW: single spot R_f ca. 0.55.
^{1}H-NMR is compatible with the structure, chemical shifts in ppm: tert. butyl (Boc) 1.1, CHα 4.03, CH_2 4.23, CH_2 (Fmoc) 4.28, NH 6.95, 7.35, aromat H (Fmoc) 7.4, 7.68, 7.9.

Example 4.3: Synthesis of Fmoc-D-Asp(OBut)-Trp(Boc)-TAB-amide-resin

In analogy to example 1.1, the compound is synthesized starting from 1.36 g Fmoc-TAB-amide-resin (Novabiochem) (0.60 mmol) by sequential coupling of Fmoc-Trp(Boc) and Fmoc-D-Asp(OBut) as described in example 1.1. The resulting resin is dried under reduced pressure: 4.7 g.

Example 4.4: Cleavage of Fmoc-D-Asp-Trp-amide from the TAB-amide-resin and subsequent removal of the tert-butyl protecting groups

4.7 g Fmoc-D-Asp(OBut)-Trp(Boc)-TAB-amide-resin (1.79 mmol) is treated 6 times with 15 ml of a mixture of TFA 95 % and DCE (1:1 v/v) containing 2 % ethanedithiole for 5 minutes. The resin is filtrated and washed as follows: 3 times with DCE (15 ml each) and 3 times with TFE (15 ml each). The combined filtrates are concentrated under reduced pressure at 25-30°C to a volume of 10 ml and added under stirring to a mixture of 250 ml DIPE and PE (1:1 v/v). The precipitate is filtered and, after washing with the precipitation mixture (30 ml), dried under reduced pressure: 0.883 g.

For complete removal of the tert-butyl protecting groups, the colorless solid is treated with a mixture of 20 ml TFA and ethanedithiole (98:2 v/v) for 20 minutes. The solution is concentrated to ca. 10-15 ml and the product is precipitated and isolated as described above. For complete decarboxylation of the carboxy-N-indolyl intermediate the crude material (ca. 0.836 g) is dissolved in 120 ml of a mixture of CH_3CN and DMF (1:1 v/v) and kept for 15 hours. After concentration under reduced pressure, the product is precipitated as described above, and dried under reduced pressure: 0.663 g.

Tlc CMW: single spot R_f ca. 0.5.

Analytical HPLC as described, retention time 24.0 min.
MALDI, positive mode, 539.9 (calculated: 541.6).

Example 4.5: Coupling of the Fmoc-D-Asp-Trp-amid to the TAB-ester-resin via the Asp-side chain.

0.660 g Fmoc-D-Asp-Trp-amid (1.22 mmol) are dissolved in 2 ml DMA (dest.), 10 ml DCE and 2.10 g TAB-resin (Novabiochem) (1.22 mmol) are added under slow stirring. The mixture is cooled to 0-5°C and a solution of 0.503 g DCCI (2.44 mmol) in 1 ml DMA and 0.3 ml DCE is added. After 5 minutes at 0-5°C a solution of 29.8 mg DMAP (0.244 mmol) in 2 ml DCE is added. After 20 minutes at 0-5°C 135.6 µl N-methylmorpholine is added. The reaction mixture is then shaken for 4 hours. The resin is washed as follows: 6 times with DMA (30 ml each), 5 times with isopropanole (30 ml each). The resin is dried under reduced pressure: 2.5 g (with traces of solvents).

Example 4.6: Synthesis of the protected resin-bound peptide Boc-Dpr-Arg(Pmc)-Ser(But)-D-Asp(TAB-ester-resin)-Trp-amid

2.5 g Fmoc-D-Asp(TAB-ester-resin)-Trp-amid (1.22 mmol) is subjected to a solid-phase synthesis process analogues to example 1.1, attaching in a sequential manner the following amino acid derivatives: Fmoc-Ser(But), Fmoc-Arg(Pmc) and Boc-Dpr(Fmoc). At the end of the synthesis the Fmoc-group at the Dpr-side chain is removed (20% solution of piperidine in DMA, see example 1.1) and the resin is dried under reduced pressure: ca. 3.0 g (with traces of solvents).

Example 4.7: Cleavage of the linear, protected peptide from the TAB-ester-resin

647 µmol peptide-resin (ca. 3.0 g, containing solvents) is shaken with 50 ml of a mixture of 99 % AcOH and DCM (1:9 v/v) for 1.5 hours. The resin is filtered and washed twice with DCE (40 ml each), once with a mixture of DCE and TFE (1:1 v/v) (40 ml) and four times with TFE (40 ml each). The combined filtrates are concentrated under reduced pressure at 25-30°C and the oily residue is dissolved in ca. 100 ml DMSO (distilled) and lyophilized. For complete removal of traces of AcOH which would interfere with the

following process of cyclization, two additional lyophilizations are applied to the product (from 25 ml DMSO each): 0.704 mg (with traces of solvents).
Analytical HPLC as described, retention time 23.9 min.
MALDI, positive mode, 1091.1 (calculated 1089.3).

Example 4.8: Cyclization of the protected linear peptide
Boc-Dpr-Arg(Pmc)-Ser(But)-D-Asp-Trp-amid

0.874 g HOBt (6.47 mmol) and 1.33 g DCCI (6.47 mmol) are dissolved in 162 ml DMF (degassed under reduced pressure). To this solution, 0.704 g linear peptide (0.647 mmol) dissolved in 162 ml DMF, is added under stirring during 9.5 hours and then reacted for 15 hours. The reaction mixture is concentrated at reduced pressure to a volume of about 5 ml, precipitated dicyclohexyl-urea is removed by filtration, and the filtrate is added to 120 ml DIPE. After 30 minutes at 0°C, the resulting precipitation is filtrated and washed with 50 ml DIPE and dried under reduced pressure (0.649 g).

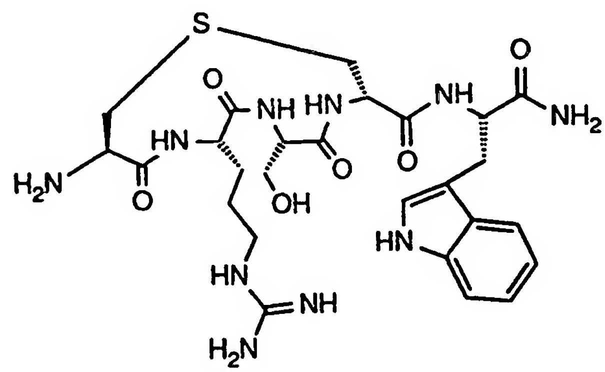
Analytical HPLC as described, retention time 26.8 min.

Example 4.9: Cleavage of the protecting groups at the cyclic peptide

56.7 mg crude protected cyclic peptide is dissolved in 1 ml of a mixture of TFA 95% and ethanedithiole (9:1 v/v) and reacted for 2 hours. The crude peptide is precipitated by the addition of 10 ml of a 1:1 mixture (v/v) of DIPE and petroleum ether (low-boiling). The precipitate is isolated by filtration, washed with 3 ml of the precipitation mixture and dried under reduced pressure. The crude material is purified with HPLC (retention time 10.0 minutes) and converted to the acetate as described in example 1.3. The compound of the formula (6) is obtained as a fluffy white powder.

Analytics:
analytical HPLC as described, retention time of the single peak is 11.0 minutes.
MALDI, positive mode: 628.3 (calculated mass: 630.7).

Example 5: Synthesis of a compound of the formula (7)



(7)

Example 5.1: Boc-Cys(Acm)-Arg(Pmc)-Ser(But)-D-Cys(Trt)-Trp(Boc)-'Wang'-resin

The solid-phase attached protected peptide is synthesized in analogy to example 1.1 by sequential coupling of Fmoc-D-Cys(Trt), Fmoc-Ser(But), Fmoc-Arg(Pmc) and Boc-Cys(Acm).

Example 5.2: Disulfide cyclization of the peptide on the resin

0.8 g Peptide-resin (0.145 mmol) previously treated with isopropanole, is shaken in a solution of 185 mg iodine (0.725 mmol) in 1.5 ml ethanol and 1.5 ml chloroform. After 7 minutes, 10 µl DIPEA are added for neutralization of HI. After a total reaction time of 15 minutes, the resin is filtered and washed as follows: 5 times with ethanol, 5 times with chloroform, 5 times with DMA (10 ml each). The resin is then treated twice with a solution of 25 mg ascorbic acid in 100 µl water and 4.9 ml ethanol for 1 minute. Extensive washings are then applied (three times DMA and isopropanole (10 ml)) and the resin is dried at reduced temperature: 0.8 g.

An analytical sample is cleaved from the resin as described in example 1.2 and demonstrated that the expected cyclic disulfide peptide has been formed.

MALDI, positive mode, 652.5 (calculated: 652.7).

Example 5.3: Desulfurization of the cyclic disulfide peptide on the resin

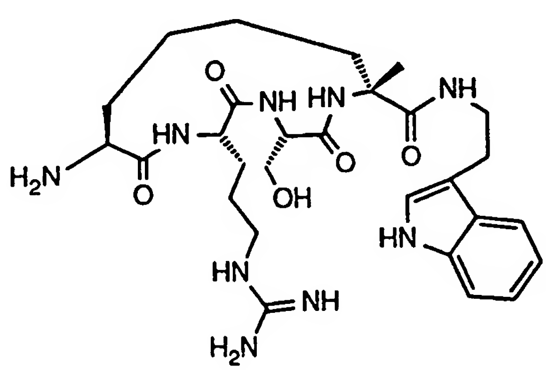
0.8 g peptide-resin (0.145 mmol) is washed 12 times with 'dry' DMA (10 ml each). A solution of 220 µl tris-(diethylamino)-phosphin (0.8 mmol) in 5 ml 'dry' DMA is added to the resin and the mixture is maintained for 48 hours under exclusion of water and slow shaking for 1 minute six times per hour. The solution is removed by filtration and the resin is washed as follows: 8 times with DMA and 5 times with isopropanol (10 ml each), and dried under reduced pressure: 0.8 g.

Example 5.4: Cleavage from the resin and the protecting groups

The cleavage at the aforementioned resin is performed in an analogous manner as described in example 1.2 to give 90 mg. Purification with HPLC is accomplished as in example 1.3, except that the gradient is running from 10% to 60% during 30 minutes. The peak with the retention time of 10.5 minutes is collected, concentrated and filtrated through an acetate ion-exchanger as described in example 1.3. After lyophilization the compound of the formula (7) is obtained as a white powder.

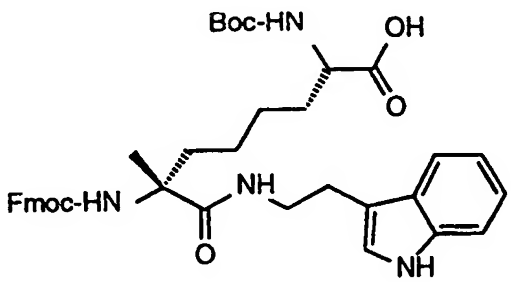
Analytical HPLC as described, retention time is 14.9 minutes.
Maldi, positive mode: 620.6 (calculated: 620.7).

Example 6: Synthesis of a compound of the formula (8)



(8)

Example 6.1: Synthesis of a compound of the formula (8.1)



(8.1)

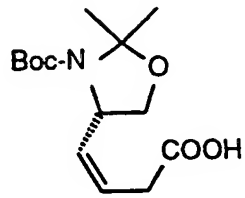
Example 6.1.1: Synthesis of 1,1.dimethylethyl-4-formyl-2,2-dimethyl-3-oxazolidine-carboxylate

1,1.dimethylethyl-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate is synthesized according to Garner et al., J. Org. Chem. (1987), 52, 2361-2364 starting from D-serine.

^{1}H-NMR ($CDCl_3$): 9.52 (s, 1H); 4.20 (m, 1H); 4.08 (m, 2H); 1.62 (s, 3H); 1.55 (s, 3H); 1.47 (s, 9H).

Example 6.1.2: Addition of a carboxyalkene group

An equimolar amount of triphenylphosphine and 3-bromopropionic acid are dissolved in toulene and refluxed under argon for 16-20 hours. The cooled reaction mixture is filtrated, washed with ether and dried under vacuum. 3.89 g (9.07 mmol) of the phosphonium salt are suspended in 50 ml dry THF in a three-necked flask under an argon atmosphere. The suspension is cooled to -75°C, and n-butyllithium (9.07 mmol, 1.3 M in hexane) is added dropwise. The mixture is stirred for 0.5 h, allowing the temperature to reach 0°C at which point it is stirred for additional 1 hour. This suspension is cooled to -75°C and 1.22 g (5.34 mmol) of the aldehyde according to example 6.1.1 is added and the reaction is allowed to proceed for 30 min. at 0°C and 1 hour at room temperature. The compound of the formula (8.2) is obtained as a clear oil (P.L. Beaulieu. et al., J. Org. Chem. (1991), 56, 4196-4204).



(8.2)

^{1}H-NMR ($CDCl_3$): 5.49 (m, 2H); 4.08 (dd, 1H); 2.67 (m, 1H); 2.45 (m, 4H); 1.59 (s, 3H); 1.52 (s, 3H); 1.47 (s, 9H).

Example 6.1.3: Methylation of the carboxy group

To a solution of 0.91 g of the compound of the formula (8.2) in methylene chloride are added 0.72 g of dicyclohexylcarbodiimide, 0.3 ml of MeOH and 0.04 g of DMAP. The reaction mixture is stirred for 3 hours at room temperature, filtered and the filtrate concentrated in vacuo. Purification of the residue by flash chromatography (methylene chloride/methanol 10:0.1) yields the pure methyl-ester as a colorless oil.

^{1}H-NMR ($CDCl_3$): 5.65 (m, 2H); 4.60 (m, 1H); 4.10 (dd, 2H); 3.70 (s, 3H); 3.25 (m, 2H); 1.59 (s, 3H); 1.52 (s, 3H); 1.47 (s, 9H).

Example 6.1.4: Reduction of the ester group

To a solution of 0.76 g of the methyl-ester in methylene chloride is added dropwise 4.7 ml of 1.2 M diisobutylaluminum hydride (DIBAH) in toluene at 0°C. The reaction mixture is stirred at 0°C for 1 hour and 10 ml of 1N HCl is added dropwise. The mixture is poured on H_2O and the organic layer separated, dried over $CaCl_2$ and concentrated in vacuo. Purification of the residue by flash chromatography (ethyl acetate/hexane 7:3) yields the alcohol compound as a colorless oil.

^{1}H-NMR ($CDCl_3$): 5.55 (m, 2H); 4.70 (m, 1H); 4.10 (dd, 2H); 3.80-3.50 (m, 4H); 1.52 (s 6H); 1.42 (s, 9H).

Example 6.1.5: Tosylation of the alcohol group

To a solution of 0.680 g of the alcohol in methylene chloride are added 0.45 ml of $(CH_3CH_2)_3N$; 0.52 g of toluene-4-sulfonyl chloride and 0.04 g of dimethylamino pyridine at 0°C. The ice-bath is immediately removed and the reaction mixture stirred for 4 hours at room temperature. The reaction mixture is poured on ice and extracted with methylene chloride in two portions. The combined organic extracts are dried over $CaCl_2$ and concentrated in vacuo. The residue is purified by flash chromatography (hexane/ethyl acetate 6:1).

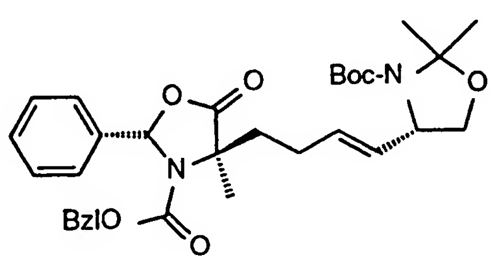
^{1}H-NMR ($CDCl_3$): 7.8 (d, 2H); 7.35 (d, 2H); 5.55 (m, 1H); 5.40 (m, 1H); 4.53 (m, 1H); 4.18-3.90 (m, 4H); 3.60 (dd, 1H); 2.60 (dd, 1H); 2.47 (s, 3H); 1.59 (s, 3H); 1.52 (s, 3H); 1.47 (d, 9H).

Example 6.1.6: Replacement of the tosyl group by iodine

To a solution of 0.535 g of the tosylate in DMF is added 0.675 g of LiI and the reaction mixture is warmed to 70°C for 20 min. The reaction mixture is partitioned between H_2O and ethyl acetate, the organic layer is separated, dried over sodium sulfate and concentrated in vacuo. The residue is purified by flash chromatography (hexane/ethyl acetate 7:1) to yield pure iodinated compound as a slightly yellow oil.

^{1}H-NMR ($CDCl_3$): 5,55 (m, 1H); 5.45 (m, 1H); 4.6 (m, 1H); 4.1 (dd, 1H); 3.70 (dd, 1H); 3.30 (m, 1H); 3.10 (m, 1H); 2.75 (m, 2H); 1.59 (s, 3H); 1.52 (S, 3H); 1.47 (s, 9H).

Example 6.1.7: Synthesis of a compound of the formula (8.3)



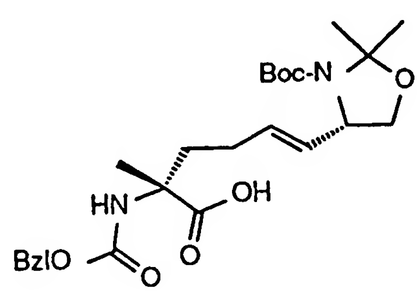
(8.3)

A solution of 0.17 ml of 1,1,1,3,3,3-hexamethyldisilazane in 0.5 ml of THF is cooled to -78°C, and 0.59 ml of 1.6 M butyllithium is added. The resulting solution is stirred for 10 min at -78°C and then transferred to a precooled solution (-78°C) of 0.232 g of (2S,4R)-3-[(benzyloxy)carbonyl]-4-methyl-2-phenyl-1,3-oxazol in-5-one (Altmann et. al., Helv. Chim. Acta (1991), 74, 800-806) in 0.8 ml of THF. The sightly yellow enolate solution is stirred for 5 min at -78°C and then 0.568 g of the iodinated compound according to example 6.1.6, dissolved in 1 ml of THF is added. The reaction mixture is warmed to -40°C and stirred at this temperature for 4 hours, then partitioned between saturated ammonium chloride solution and ether, the aq. layer is separated and extracted twice with ether, the combined ether extracts are dried over sodium sulfate and concentrated in vacuo. Purification of the residue is carried out by flash chromatography (hexane/ether 8:2).

^{1}H-NMR($C_2D_2Cl_4$; 100°C): 7.36-7.10 (m, 10H); 6.48 (s, 1H); 5.40 (m, 1H); 5.30 (m, 1H); 4.50 (m, 1H); 4.00 (dd, 1H); 3.60 (dd, 1H); 2.40 (m, 1H); 2.10 (m, 1H); 2.00 (m, 2H); 1.70 (s, 3H); 1.59 (s, 3H); 1.52 (s, 3H); 1.45 (s, 9H).

Example 6.1.8: Removal of the benzyl group

To a solution of 0.232 g of the compound of the formula (8.3) in 3 ml of methanol is added 0.5 ml of a 2n aq. LiOH-solution. The reaction mixture is stirred for 2 hours at 45°C, diluted with H_2O and twice extracted with ether. The combined ether extracts are dried over sodium sulfate and concentrated in vacuo. The residue is purified by flash chromatography (10:0.4 10:1, methylene chloride/methanol) and yields the compound of the formula (8.4) as a white foam.



(8.4)

^{1}H-NMR($CDCl_3$): 7.30 (s, 5H); 6.72 (s, 1H); 5.35 (m, 2H); 5.20 (d, 2H); 4.70 (m, 1H); 3.95 (dd, 1H); 3.58 (dd, 1H); 2.40-1.90 (m, 4H); 1.59 (s, 3H); 1.52 (s, 3H); 1.48 (s, 9H).

Example 6.1.9: Coupling of tryptamine to the free carboxyl group

To a solution of 0.150 g of the compound of the formula (8.4) in methylene chloride are added 74.5 mg of DCC, 55.3 mg of HOBt and 58 mg of tryptamine. The reaction mixture is stirred at room temperature for 2 hours, is filtered, diluted with methylene chloride and washed with brine. The organic layer is dried over calcium chloride and concentrated in vacuo. Purification of the residue by flash chromatography (200:5, methylene chloride/methanol) yields a white foam.

^{1}H-NMR($CDCl_3$): 8.08 (s, 1H); 7.58 (d, 1H); 7.35 (s,5H); 7.25-7.00 (m,3H); 6.58 (s, 1H); 5.35 (d, 1H); 5.30 (s, 1H); 5.08 (d, 2H); 4.50 (m, 1H); 3.88-3.50 (m,3H); 2.99 (t, 2H); 2.38-1.55 (m, 4H); 1.50 (s, 6H); 1.45 (s, 3H);1.42-1.22 (m, 2H); 1.39 (s, 9H).

Example 6.1.10: Removal of the Bzl-O-CO- protecting group

To a solution of 0.200 g of the compound according to example 6.1.9 in 8 ml of THF is added 0.030 g of 10 % palladium on charcoal under an atmosphere of nitrogen and the resulting mixture is hydrogenated 3 hours at room temperature. The catalyst is removed by filtration followed by evaporation of the solvent, which yields the free amine as a white foam.

^{1}H-NMR($CDCl_3$): 8.92 (s, 1H); 7.65 (d, 1H); 7.52 (s, 1H); 7.40 (d, 1H); 7.2-7.1 (m, 2H); 7.08 (s, 1H); 3.90 (dd, 1H); 3.85 (m, 1H); 3.80 (m, 1H); 3.72 (dd, 1H); 3.52 (m, 1H); 3.00 (t, 2H); 1.90- 1.40 (m, 6H); 1.60 (s, 3H); 1.52 (s, 6H); 1.45 (s, 9H); 1.30 (s, 2H); 1.25-1.10 (m, 2H).

Example 6.1.11: Protection of the free amino group with Fmoc

To a solution of 0.150 g of the free amine in THF are added 0.054 ml of N-ethyldiisopropylamine and 0.081 g of Fmoc-Cl and the reaction mixture is stirred at room temperature over night. The reaction mixture is partitioned between methylene chloride and brine, the organic layer separated, dried over $CaCl_2$ and the solvent

concentrated in vacuo. The residue is purified by flash chromatography (8:2 to 9:1, ether/hexane) to the Fmoc-protected compound as a white foam.

^{1}H-NMR($CDCl_3$): 8.92 (s, 1H); 7.89 (d, 2H); 7.60 (d, 2H); 7.45-7.30 (m,2H); 7.20 (t,1H); 7.12 (t, 1H); 7.00 (s, 1H), 6.28 (s, 1H); 5.85 (s, 1H); 4.35 (d, 2H); 4.15 (t, 1H); 3.83 (m, 2H); 3.65 (m, 3H); 3.00 (t, 2H); 1.80-1.00 (m, 8H); 1.58 (s, 3H); 1.55 (s, 3H); 1.50 (s , 3H); 1.46 (s, 9H).

Example 6.1.12: Removal of the acetylating cap

To a solution of 0.180 g of the Fmoc-protected compound in methanol/H_2O (95:5) is added 2 mg of toluene-4-sulfonic acid and the reaction mixture is warmed to 75°C for 5 hours. The reaction mixture is concentrated in vacuo and the residue is purified by flash chromatography (200:5→100:5 ; ether/methanol) to give the free alcohol.

^{1}H-NMR($CDCl_3$): 8.92 (s, 1H);7.89 (d, 2H); 7.60 (d, 2H); 7.45-7.30 (m,2H); 7.20 (t,1H); 7.12 (t, 1H); 7.00 (s, 1H), 6.28 (s, 1H); 5.85 (s, 1H); 4.35 (d, 2H); 4.15 (t, 1H); 3.83 (m, 3H); 3.65 (m, 2H); 3.00 (t, 2H); 1.80-1.00 (m, 8H); 1.62 (s, 3H); 1.46 (s, 9H).

Example 6.1.13: Oxidation of the free hydroxy group

To a solution of 0.100 g of the free alcohol in DMF is added 0.112 g of pyridinium dichromate. The reaction mixture is stirred at room temperature for 5 hours and then partitioned between ethyl acetate and brine. The organic layer is separated, dried over sodium sulfate and concentrated in vacuo. The residue is purified by flash chromatography (10:1 ; methylene chloride/methanol) to yield the pure product of the formula (8.1).

^{1}H-NMR($CDCl_3$): 8.92 (s, 1H);7.89 (d, 2H); 7.60 (d, 2H); 7.45-7.30 (m,2H); 7.20 (t,1H); 7.12 (t, 1H); 7.00 (s, 1H), 6.28 (s, 1H); 5.85 (s, 1H); 4.48 (m, 1H); 4.35 (d, 2H); 4.15 (t, 1H); 3.66 (m, 2H); 3.08 (t, 2H); 1.92-1.00 (m, 8H); 1.62 (s, 3H); 1.47 (s, 9H).

Example 6.2: Coupling of the compound of the formula (8.1) to the TAB-ester-resin

The compound of the formula (8.1) is attached to the resin in an analogue manner as described in example 4.5.

Example 6.3: Synthesis of the protected resin-bound peptide Arg(Pmc)-Ser(But)-(8.1)-(TAB-ester-resin)

1.5 g of the compound according to example 6.2 (0.7 mmol) is subjected to a solid-phase synthesis process analogues to example 1.1 attaching in a sequential manner the following amino acid derivatives: Fmoc-Ser(But) and Fmoc-Arg(Pmc). At the end of the synthesis the Fmoc-group at the Arg is removed and the resin is dried under reduced pressure: ca. 1.9 g (contains traces of solvents).

Example 6.4: Cleavage of the linear, protected peptide from the TAB-ester-resin

Ca. 1.9 g peptide-resin (ca. 0.65 mMol) is shaken with 30 ml of a mixture of 99 % AcOH and DCM (1:9 v/v) for 1.5 hours. The resin is filtered and washed twice with DCE (20 ml each), once with a mixture of DCE and TFE (1:1 v/v) (20 ml) and four times with TFE (20 ml each). The combined filtrates are concentrated under reduced pressure at 25-30°C and the oily residue is dissolved in ca. 50 ml DMSO (distilled) and lyophilized. For complete removal of traces of AcOH which would interfere with the following process of cyclization, two additional lyophilizations are applied to the product (from 10 ml DMSO each): 0.48 g.

Analytical HPLC as described, retention time 26.3 min.
MALDI, positive mode, 1027.1 (calculated 1027.3).

Example 6.5: Cyclization of the protected linear peptide

0.48 g linear peptide according to 8.4 (0.467 mMol) is cyclisized in an analogue manner as described in example 4.9. Analytical HPLC as described, retention time 27.2 min.

MALDI, positive mode, 1009.0 (calculated 1009.2).

Example 6.6: Cleavage of the protecting groups at the cyclic peptide

180 mg crude protected cyclic peptide is dissolved in 2 ml of a mixture of TFA 95 % and ethanedithiole (9:1 v/v) and reacted for 2 hours. The crude peptide is precipitated by the addition of 15 ml of a 1:1 mixture (v/v) of DIPE and petroleum ether (low-boiling). The precipitate is isolated by filtration; washed with 5 ml of the precipitation mixture and dried under reduced pressure. The crude material is purified with HPLC (retention time 12.3 minutes) and converted to the acetate as described in example 1.3. The compound of the formula (8) is obtained as a fluffy white powder.

Analytical HPLC as described, retention time of the single peak is 12.0 minutes.
MALDI, positive mode: 586.1 (calculated mass: 586.7).

Example 6.7: Alternativ pathway for the synthesis of a compound of the formula (8)

Example 6.7.1: Opening of the oxazolidine in the compound of formula (8.3)

To a solution of 1.20 g of the compound of formula (8.3) (Example 6.1.7) in methanol/H_2O (95:5) is added 0.060 g of toluene-4-sulfonic acid and the reaction mixture is stirred at 75°C for 7 hours. The reaction mixture is concentrated in vacuo and the residue is purified by flash-chromatography (2:1, ether:hexane) to give the pure product as a white foam.
^{1}H-NMR($CDCl_3$): 7.40 (m, 6H); 7.20 (m, 2H); 6.90 (m, 2H); 6.50 (s, 1H); 5.60 (m, 1H); 5.31 (m, 1H); 5.08 (m, 2H); 4.70 (d, 1H); 4.39 (m, 1H); 3.57 (m, 2H); 2.60 (m, 1H); 2.22 (m, 1H); 2.02 (m, 2H); 1.79 (s, 3H); 1.71 (m, 1H); 1.48 (s, 9H).

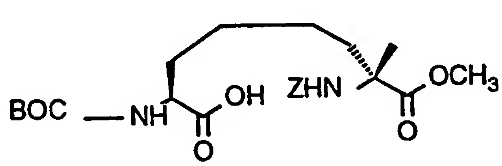
Example 6.7.2: Oxidation of the OH-group

A solution of 0.600 g of the compound according to example 6.7.1 in 14 ml of acetone is cooled to 0°C and 0.90 ml of Jones-reagent (3.25 M CrO_3/5.29 M H_2SO_4) is added

dropwise. The reaction mixture is stirred at 0°C for two hours, then 1.3 ml of isopropanol is added dropwise. The reaction mixture is filtered and the filtrate is partitioned between ethyl acetate and brine. The aq. layer is extracted three times with ethyl acetate. The combined organic extracts are washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue is purified by flash-chromatography (10:0.5; methylene chloride:methanol) to give the pure product as a white foam.
^{1}H-NMR($CDCl_3$):7.40 (m, 6H); 7.20 (m, 2H); 6.85 (m, 2H); 6.54 (s, 1H); 5.55 (m, 1H); 5.33 (m, 1H); 5.08 (m, 2H); 4.82 (m, 1H); 4.39 (m, 1H); 2.24 (m, 1H); 2.05 (m, 2H); 1.82 (s, 3H); 1.75 (m, 1H); 1.48 (s, 9H).

Example 6.7.3: Opening of the oxazolidone

To a solution of 1.10 g of the compound according to example 6.7.2 in 17 ml of methanol is added 0.09 g of LiOH dissolved in 1.0 ml of H_2O. The reaction mixture is stirred for 30 min at room temperature, then partitioned between H_2O/ether. The aq. layer is separated and acidified with 1 N HCl and again extracted with ether. The acidic ether extracts are combined and dried over sodium sulfate. Evaporation of the solvent yields the pure product of formula (8.5) as a white foam.
^{1}H-NMR(CD_3OD): 7.30 (s, 5H); 6.12 (m, 1H); 5.10 (m, 2H); 5.25 (m, 1H) ; 5.10 (m, 2H); 3.80 (s, 3H); 2.30-2.10 (m, 3H); 1.60 (s, 3H); 1.42 (s, 9H); 1.28 (t, 2H).



(8.5)

Example 6.7.4: Introduction of a 2-trimethylsilyl ethyl protecting group

To a solution of 0.74 g of (8.5) in 15 ml of methylene chloride are added 0.36 g of dicyclohexylcarbodiimide, 0.019 g of dimethylaminopyridine and 0.23 ml of 2-trimethylsilyl ethanol. The reaction mixture is kept for 18 hours at room temperature; it is then filtered and the filtrate is evaporated. The residue is purified by flash-chromatography (1:1, ether:hexane) to give the pure product as a colorless oil.
^{1}H-NMR($CDCl_3$):7.36 (m, 5H); 6.21 (s (broad), 1H); 5.59 (m, 1H); 5.20 (m, 1H); 5.10 (s,

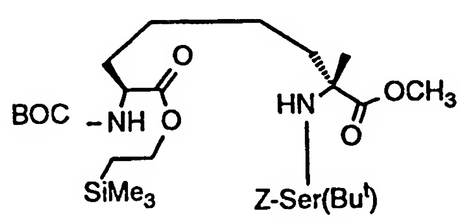
2H); 5.02 (d, 1H); 4.20 (t, 2H); 3.75 (m, 1H); 3.70 (s, 3H); 2.00-1.90 (m, 1H); 1.60 (s, 3H); 1.40 (s, 9H); 1.20 (t, 2H); 0.99 (t, 2H); 0.20 (s, 9H).

Example 6.7.5: Removal of the Z-protecting group

0.780 g of the compound according to example 6.7.4 is hydrogenated over 0.150 g of Pd/C (10%) in 40 ml of tetrahydrofurane. The catalyst is removed by filtration and the filtrate is evaporated in vacuo to give the pure product as a colorless oil.
^{1}H-NMR($CDCl_3$):5.02 (d, 1H); 4.21 (t, 2H); 4.10 (m, 1H); 3.80 (s, 3H); 1.90-1.10 (m, 6H); 1.60 (s, 3H); 1.40 (s, 9H); 1.28 (t, 2H); 1.00 (t, 2H); 0.20 (s, 9H).

Example 6.7.6: Condensation with Z-Ser(Bu^t)-OH

To a solution of 0.400 g of the compound according to example 6.7.5 in 13 ml of tetrahydrofurane are added 0.872 g of Z-Ser(Bu^t)-OSu and 0.31 ml of Et_3N. The reaction mixture is stirred for 17 hours at room temperature. The solvent is then evaporated and the residue is purified by flash-chromatography (10:0.5, ether:methanol) to give the pure product of formula (8.6).
FAB-MS : 709 $(M+H)^+$.
^{1}H-NMR(DMSO; 80°C):7.38-7.29 (m, 5H); 5.07 (s, 2H); 4.13 (m, 3H); 3.90 (m, 1H); 3.59 (s, 3H); 3.47 (d, 2H); 1.77 (m, 2H); 1.60 (m, 2H); 1.39 (s, 9H); 1.38 (s, 3H); 1.35-1.16 (m, 4H); 1.14 (s, 9H); 0.95 (t, 2H); 0.04 (s, 9H).



(8.6)

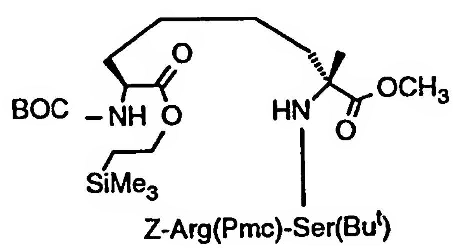
Example 6.7.7: Removal of the Z-protecting group

0.639 g of (8.6) is hydrogenated over 0.220 g of Pd/C (10%) in 40 ml of tetrahydrofurane. The catalyst is removed by filtration and the filtrate is evaporated to give the pure product.

^{1}H-NMR($CDCl_3$): 5.00 (d(broad), 1H); 4.21(m, 3H); 3.82 (s, 3H); 3.50 (m, 3H); 2,20 (m, 1H); 1.80 (m, 1H); 1.65 (s, 2H); 1.58 (s, 3H); 1.45 (s, 9H); 1.40-1.10 (m, 4H); 1.20 (s, 9H); 1.00 (t, 2H); 0.30 (s, 9H).

Example 6.7.8: Condensation with Z-Arg(Pmc)-OH

To a solution of 0.639 g of Z-Arg(Pmc)-OH in 15 ml of tetrahydrofurane are added at 0°C 0.439 g of benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium-hexafluoro-phosphate (BOP) and 0.14 ml of Et_3N. The reaction mixture is stirred for 5 min and then 0.584 g of the compound according to example 6.7.7 is added. The ice-bath is removed and the reaction mixture is stirred for 2 hours at room temperature. The reaction mixture is then poured into saturated NH_4Cl-solution and the aq. layer is three times extracted with ether. The combined organic extracts are washed with H_2O and brine, dried over sodium sulfate and the solvent is evaporated. The residue is purified by flash-chromatography (10:0.3, methylene chloride:methanol) to give the pure product of formula (8.7) as a foam.
FAB-MS : 1132 $(M+H)^+$.
^{1}H-NMR(DMSO; 120°C): 7.37-7,27 (m, 5H); 5.06 (s, 2H); 4.33 (m, 1H); 4.16 (t, 2H); 4.04 (m, 1H); 3.93 (m, 1H); 3.60 (s, 3H); 3.53 (m, 1H); 3.43 (m, 1H); 3.08 (q, 2H); 2.62 (t, 2H); 2.53 (s, 3H); 2.52 (s, 3H); 2.06 (s, 3H); 1.81 (t, 2H); 1.78-1.18 (m, 12H); 1.42 (s, 3H); 1.40 (s, 9H); 1.30 (s, 6H); 1.15 (s, 9H); 0.97 (t, 2H); 0.05 (s, 9H).



(8.7)

Example 6.7.9: Removal of the 2-trimethylsilyl ethyl protecting group

To a solution of 1.045 g of (8.7) in 18 ml of tetrahydrofurane is added 0.584 g of tetrabutylammoniumfluoride. The reaction mixture is stirred for 24 hours at room temperature and is then poured into saturated NH_4Cl-solution. The aq. layer is three times extracted with ethyl acetate. The combined organic extracts are washed with H_2O and

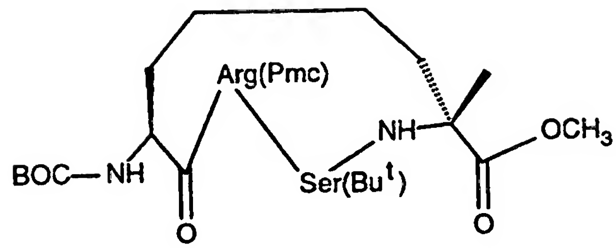
brine and the solvent is evaporated in vacuo. Purification of the residue by flash-chromatography (10:0.8, methylene chloride:methanol) yields the pure product as a white foam.
FAB-MS: 1032 $(M+H)^+$.
^1H-NMR(DMSO;100°C):7.30-7.25 (m, 5H); 5.06 (s,2H); 4.33 (m, 1H); 4.06 (m, 1H); 3.65 (m, 1H); 3.59 (s, 3H); 3.53 (m, 1H); 3.43 (m, 1H); 3.08 (q, 2H); 2.62 (t, 2H); 2.53 (s, 3H); 2.52 (s, 3H); 2.06 (s, 3H); 1.80 (t, 2H); 1.78-1.20 (m, 12H); 1.42 (s, 3H); 1.40 (s, 9H); 1.30 (s, 6H); 1.15 (s, 9H).

Example 6.7.10: Removal of the Z-protecting group

0.697 g of the compound according to example 6.7.9 is hydrogenated over 0.136 g of Pd/C (10%) in 40 ml of methanol. The catalyst is removed by filtration and the filtrate is evaporated to give the pure product as a white foam.
FAB-MS : 898 $(M+H)^+$.
^1H-NMR(CD_3OD): 4.47 (m, 1H); 3.95 (m, 1H); 3.65 (m, 1H); 3.59 (s, 5H); 3.20 (m, 2H); 2.68 (t, 2H); 2.53 (s, 3H); 2.52 (s, 3H); 2.10 (s, 3H); 1.90-1.20 (m, 12H); 1.82 (t, 2H); 1.48 (s, 3H); 1.42 (s, 9H); 1.30 (s, 6H); 1.20 (s, 9H).

Example 6.7.11: Cyclization

To a solution of 0.341 g of 1-hydroxybenzotriazole and 0.459 g of dicyclohexylcarbodiimid in 56 ml of dimethylformamide is added dropwise within 9.5 hours a solution of 0.200 g of the compound according to example 6.7.10 dissolved in 56 ml of dimethylformamide. The reaction mixture is stirred at room temperature for 16 hours, then the solvent is evaporated in vacuo and the residue is purified by flash-chromatography (10:0.6, methylene chloride:methanol) to give the pure product of formula (8.8) as a white powder.
FAB-MS : 881 $(M+H)^+$.
^1H-NMR(CD_3OD): 4.32 (m, 1H); 4.21 (m, 1H); 3.90 (m, 1H); 3.78 (s, 3H); 3.57 (m, 2H); 3.19 (m, 2H); 2.78 (t, 2H); 2.58 (s, 3H); 2.56 (s, 3H); 2.10 (s, 3H); 1.82 (t, 2H); 1.73-1.20 (m, 10H); 1.42 (s, 3H); 1.38 (s, 9H); 1.30 (s, 9H); 1.18 (m, 8H).



(8.8)

Example 6.7.12: Cleavage of the methyl ester

To a solution of 0.087 g of (8.8) in 2 ml of methanol 0.12 ml of 1 N NaOH is added. The reaction mixture is stirred for 20 hours at 45-50°C, then diluted with H_2O and the aq. layer is once extracted with ether. The aq. layer is acidified with 1% $KHSO_4$-solution and again extracted with ethyl acetate. The combined ethyl acetate extracts are washed with brine and dried over sodium sulfate. Evaporation of the solvent yields the pure product as a white powder.

FAB-MS : 866 $(M+H)^+$.

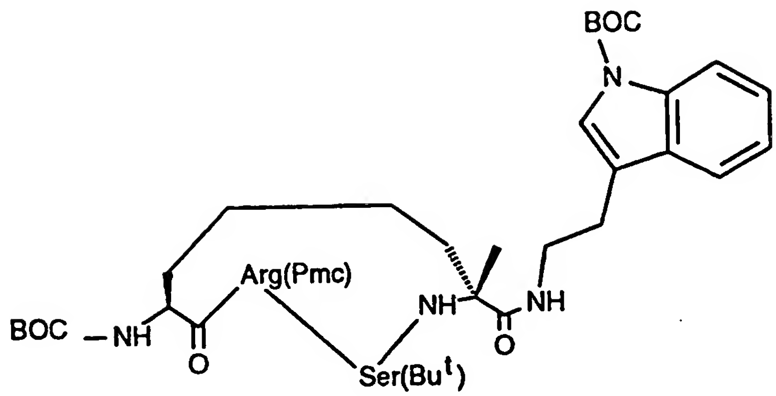
^{1}H-NMR(CD_3OD): 4.32 (m, 1H); 4.18 (m, 1H); 3.90 (m, 1H); 3.60 (m, 2H); 3.20 (m, 2H); 2.68 (t, 2H), 2.57 (s, 3H); 2.56 (s, 3H); 2.10 (s, 3H); 1.80 (t, 2H); 1.80-1.20 (m, 10H); 1.43 (s, 3H); 1.38 (s, 9H); 1.30 (s,9H); 1.18 (m, 8H).

Example 6.7.13: Condensation with tryptamine

To a solution of 0.08 g of the compound according to example 6.7.12 in 2 ml of methylene chloride are added 0.021 g of dicyclohexylcarbodiimide and 0.016 g of 1-hydroxybenzotriazole. The reaction mixture is stirred for 10 min at room temperature then a solution of 16.2 mg of tryptamine in 2 ml of methylene chloride is added. The reaction mixture is stirred for 3.5 hours at room temperature and then the solvent is evaporated in vacuo. Separation of the desired product of formula (8.9) from the two diastereomeric products is achieved by flash-chromatography (methylene chloride to 10:0.5 methylene chloride:methanol).

FAB-MS : 1008 $(M+H)^+$.

^{1}H-NMR(CD_3OD) of compound of formula (8.8): 7.58 (d, 1H); 7.30 (d, 1H); 7.10-6.90 (m, 3H); 4.28 (m, 1H); 4.18 (m, 1H); 3.92 (m, 1H); 3.58 (m, 2H); 3.50 (t, 2H); 3.20 (m, 2H); 2.98 (m, 2H); 2.59 (t, 2H); 2.57 (s, 3H); 2.56 (s, 3H); 2.10 (s, 3H); 1.80 (t, 2H); 1.80-1.20 (m, 10H); 1.43 (s, 3H); 1.40 (s, 9H); 1.30 (s, 9H); 1.15 (m, 8H).

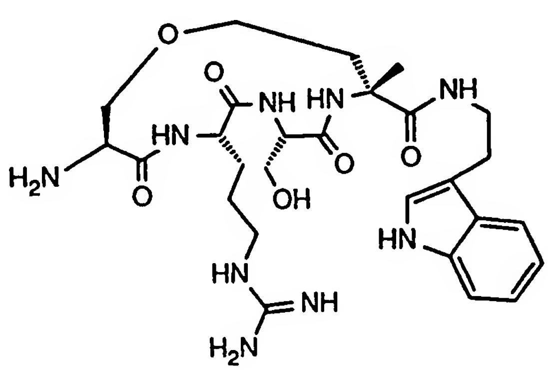


(8.9)

Example 6.7.14: Removal of the protecting groups

0.035 g of (8.9) is dissolved in 1.6 ml of trifluoroacetic acid/H_2O (90:10) and the reaction mixture is stirred for 4.5 hours at room temperature. Then the solvent is evaporated and the residue is solidified with ether. The product of formula (9) is purified by HPLC, using a Nucleosil reversed-phase C_{18} column; gradient : 10% CH_3CN (0.09% trifluoroacetic acid) / 90% H_2O (0.09% trifluoroacetic acid) to 90% CH_3CN (0.09% trifluoroacetic acid) / 10% H_2O (0.09% trifluoroacetic acid) within 30 min ;
R_t =12.00 min.
FAB-MS : 586 $(M+H)^+$.

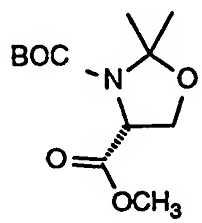
Example 7: Synthesis of a compound of the formula (9)



(9)

Example 7.1: Synthesis of 3-(1,1Dimethylethyl) 4-Methyl (R)-2,2-Dimethyl-3,4-oxazolidinecarboxylate (formula 9.1)

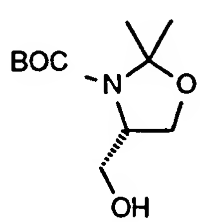
3-(1,1Dimethylethyl) 4-Methyl (R)-2,2-Dimethyl-3,4-oxazolidinecarboxylate (formula 9.1) is synthesized starting from D-serine according to Garner *et al.* J. Org. Chem. (1987), 52, 2361-2364.
^{1}H-NMR ($CDCl_3$): 4.49-4.39 (m, 1H); 4.18-4.00 (m, 2H); 3.75 (s, 3H); 1.65 (d, 3H); 1.5 (d, 3H); 1.45 (s, 9H).



(9.1)

Example 7.2: Reduction of (9.1)

A solution of 22.3 g of (9.1) in 450 ml of toluene is cooled to 0°C, and 200 ml of a 1.2 M DIBAH-solution in toluene is added dropwise. The resulting solution is stirred at 0-5°C for 1 hour and is then poured into 200 ml of 1N HCl. The aq. layer is separated and extracted three times with ethyl acetate. The combined organic extracts are dried over sodium sulfate and evaporated to give the crude product. Purification by flash chromatography (7:3 hexane:ethyl acetate) yields the pure product of formula (9.2) as a colorless oil.
^{1}H-NMR ($CDCl_3$): 4.15-3.5 (m, 6H); 1.55 (s, 3H); 1.45 (s, 12H).



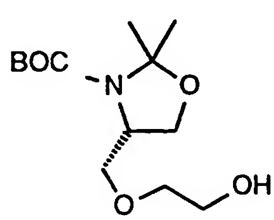
(9.2)

Example 7.3: Alkylation with methylbromoacetate

To a suspension of 2.50 g of NaH in 100 ml of tetrahydrofurane (abs.) 10.6 g of (9.2) is added dropwise. The reaction mixture is stirred for 1 hour before 14 ml of methylbromoacetate is added at once. The reaction mixture is stirred for additional 20 hours at room temperature. It is then poured into NH_4Cl-solution and the aq. layer is extracted 3 times with ether. The combined organic extracts are dried over sodium sulfate and the solvent is evaporated. Purification by flash-chromatography (3:7, ether:petrolether) gives the pure product as a colorless oil.
^{1}H-NMR ($CDCl_3$): 4.2-3.9 (m, 5H); 3.72 (s, 3H); 3.68-3.30 (m, 2H); 1.55 (s, 3H); 1.52 (s, 3H); 1.49 (s, 9H).

Example 7.4: Reduction of the methylacetate-group

A solution of 6.20 g of the compound according to example 7.3 in 120 ml of toluene is cooled to 0°C and 48.0 ml of a 1.2 M DIBAH-solution in toluene is added dropwise. The resulting solution is stirred at 0-5°C for one hour and is then poured into 150 ml of 1N HCl. The aq. layer is separated and extracted three times with ethyl acetate, the combined organic extracts are dried over sodium sulfate and concentrated in vacuo. Purification by flash-chromatography (4:6,ethyl acetate:hexane) gives the pure product of formula (9.3) as a slightly yellow oil.
^{1}H-NMR ($CDCl_3$): 4.1-3.9 (m, 3H); 3.7-3.4 (m, 7H); 1.60 (s, 3H); 1.55 (s, 3H); 1.4 (s, 9H).



(9.3)

Example 7.5: Reaction with toluene-4-sulfonylchloride

To a solution of 6.30 g of (9.3) in 100 ml of methylene chloride are added 4.1 ml of Et_3N, 4.80 g of toluene-4-sulfonylchloride and 0.28 g of dimethylaminopyridine at 0°C. The

ice-bath is immediately removed and the reaction mixture is stirred for four hours at room temperature. The reaction mixture is then poured on ice and extracted with methylene chloride in two portions. The combined organic extracts are dried over sodium sulfate and concentrated in vacuo. The residue is purified by flash-chromatography (2:8, ethyl acetate:hexane) to give the pure product as a slightly yellow oil.

^{1}H-NMR ($CDCl_3$): 7.8 (d, 2H); 7.35 (d, 2H); 4.12-3.30 (m, 9H); 2.48 (s, 3H); 1.55 (s, 3H); 1.48 (s, 12H).

Example 7.6: Replacement of the toluene-4-sulfonyl group with iodine

To a solution of 1.58 g of the compound according to example 7.5 in dimethyl formamide is added 2.17 g of LiI and the reaction mixture is stirred at 70°C for 20 min. The reaction mixture is partitioned between H_2O and ethyl acetate, the organic layer is separated, dried over sodium sulfate and concentrated in vacuo. The residue is purified by flash-chromatography (4:1, methylene chloride:hexane) to yield pure iodinated product as a slightly yellow oil.

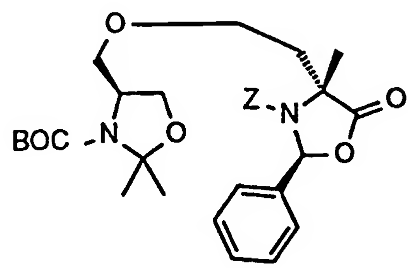
^{1}H-NMR($CDCl_3$): 4.1-3.2 (m, 9H); 1.56 (s, 3H); 1.54 (s, 3H); 1.48 (s, 9H).

Example 7.7: Replacement of iodine by (2S,4R)-3-[(benzyloxy)carbonyl]-4-methyl-2-phenyl-1,3-oxazolidin-5-one

A solution of 1.1ml of 1,1,1,3,3,3-hexamethyldisilazane in 2.1 ml of tetrahydrofurane is cooled to -78°C, and 3.20 ml of 1.6 M BuLi/hexane is added. The resulting solution is stirred for 10 min at -78°C and then transferred to a precooled solution (-78°C) of 1.45 g of (2S,4R)-3-[(benzyloxy)carbonyl]-4-methyl-2-phenyl-1,3-oxazolidin-5-one (Altmann et. al., Helv. Chim. Acta (1991), 74, 800-806) in 4.8 ml of tetrahydrofurane. The slightly yellow enolate solution is stirred for 5 min at -78°C and then 3.6 g of the compound according to example 7.6, dissolved in 6 ml of tetrahydrofurane, is added. The reaction mixture is stirred at -78°C for 24 hours and then partitioned between saturated ammonium chloride solution and ether. The aq. layer is separated, and extracted twice with ether. The combined ether extracts are dried over sodium sulfate and concentrated in vacuo. Purification of the compound of formula (9.4) is carried out by flash-chromatography (3:7, ether:hexane).

FAB-MS : 569 $(M+H)^+$.

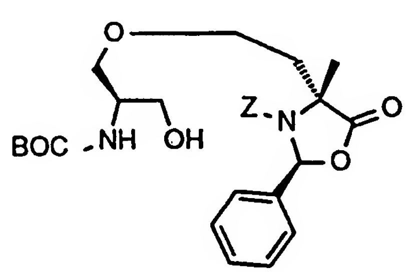
^{1}H-NMR($(CCl_2D)_2$; 80°C): 7.38 (s, 5H); 7.22 (s, 5H); 6.32 (s, 1H); 5.02 (d, 2H); 3.90 (m, 3H); 3.58 (d, 1H); 3.42 (m, 2H); 3.28 (t, 1H); 2.52 (m, 1H); 2.20 (m, 1H); 1.72 (s, 3H); 1.53 (s, 3H); 1.46 (s, 9H); 1.43 (s, 3H).



(9.4)

Example 7.8: Opening of the oxazolidine

To a solution of 1.45 g of (9.4) in methanol/H_2O (95:5) is added 73 mg of toluene-4-sulfonic acid and the reaction mixture is stirred at 75°C for 2 hours. The reaction mixture is concentrated in vacuo and the residue (formula 9.5) is purified by flash-chromatography (3:1, ether:hexane).
FAB-MS : 529 $(M+H)^+$.
^{1}H-NMR($(CCl_2D)_2$; 80°C): 7.38 (s, 5H); 7.28 (s, 5H); 6.37 (s, 1H); 5.13-4.92 (m, 3H); 3.78-3.52 (m, 4H); 3.47 (m, 3H); 2.52 (s (broad), 1H); 2.28 (m, 1H); 2.10 (m, 1H); 1.72 (s, 3H); 1.41 (s, 9H).



(9.5)

Example 7.9: Oxidation of the hydroxy group

A solution of 1.85 g of (9.5) in 48 ml of acetone is cooled to 0°C and 2.82 ml of Jones-reagent (3.25 M CrO_3/5.29 M H_2SO_4) is added dropwise. The reaction mixture is stirred at 0°C for two hours, then 4 ml of isopropanol is added dropwise. The reaction

mixture is filtered and the filtrate is partitioned between ethyl acetate and brine. The aq. layer is extracted three times with ethyl acetate. The combined organic extracts are washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue is purified by flash chromatography (10:0.75, methylene chloride:methanol) to give the pure product as a white foam.

FAB-MS : 542 $(M+H)^+$.

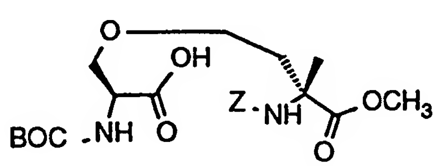
^{1}H-NMR($(CCl_2D)_2$; 80°C): 7.38 (m, 5H); 7.21 (s, 5H); 6.45 (s, 1H); 5.58 (s, 1H); 5.03 (m, 2H); 4.38 (s, 1H); 3.72 (m, 1H); 3.58 (m, 1H); 3.35 (m, 2H); 2.40 (m, 2H); 1.64 (s, 3H); 1.38 (s, 9H).

Example 7.10: Opening of the oxazolidone

To a solution of 1.65 g of the compound according to example 7.9 in 40 ml of methanol/tetrahydrofurane (1:1) is added 0.128 g of LiOH dissolved in 0.5 ml of H_2O. The reaction mixture is stirred for 1.5 hours at room temperature, then partitioned between H_2O/ether. The aq. layer is separated and acidified with 1N HCl and again extracted with ether. The acidic ether extracts are combined and dried over sodium sulfate. Evaporation of the solvent yields the pure product of formula (9.6) as a white foam.

FAB-MS : 469 $(M+H)^+$.

^{1}H-NMR(CD_3OD):7.16 (m, 5H); 5.10 (s, 2H); 4.30 (t, 1H); 3.78 (dd, 1H); 3.70 (s, 3H), 3.58 (dd, 1H); 3.50 (m, 2H); 2.18 (m, 2H); 1.50 (s, 3H); 1.43 (s, 9H).



(9.6)

Example 7.11: Protection of the free carboxyl group as 2-trimethylsilyl-ethyl ester

To a solution of 1.25 g of (9.6) in 30 ml of methylene chloride are added 0.66 g of dicyclohexylcarbodiimide, 0.032 g of dimethylaminopyridine and 0.46 ml of 2-trimethylsilyl ethanol. The reaction mixture is stirred for four hours at room temperature; it is then filtered and the filtrate is evaporated. The residue is purified by flash-chromatography (75:25, hexane:ether) to give the pure product as a slightly yellow oil.

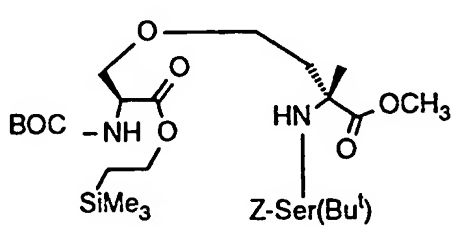
FAB-MS: 569 $(M+H)^+$.
^{1}H-NMR(CD_3OD): 7.32 (m, 5H); 5.06 (s, 2H); 4.26 (m, 1H); 4.25 (t, 2H); 3.70 (m, 1H); 3.68 (s, 3H); 3.50 (m, 4H); 2.10 (t, 2H); 1,49 (s, 3H); 1.44 (s, 9H); 1.00 (t, 2H); 0.5 (s, 9H).

Example 7.12: Removal of the Z-protecting group

1.08 g of the compound according to example 7.11 is hydrogenated over 0.360 g of Pd/C (10%) in 20 ml of tetrahydrofurane. The catalyst is removed by filtration and the filtrate is evaporated in vacuo to give the pure product.
FAB-MS : 435 $(M+H)^+$.
^{1}H-NMR($CDCl_3$): 5.68 (d, 1H); 4.33 (m, 1H); 4.20 (t, 2H); 3.85 (m, 1H); 3.72 (s, 3H); 3.50 (m, 3H); 2.10 (m, 1H); 1.78 (m, 1H); 1.70 (s, 2H); 1.47 (s, 9H); 1.32 (s, 3H); 1.00 (t, 2H); 0.50 (s, 9H).

Example 7.13: Condensation with protected serine

To a solution of 0.780 g of the compound according to example 7.12 in 25 ml of tetrahydrofurane are added 0.845 g of Z-Ser(Bu^t)-OSu and 0.40 ml of Et_3N. The reaction mixture is stirred for 40 hours at room temperature. The solvent is then evaporated and the residue is purified by flash-chromatography (1:1,ether:hexane) to give the pure product of formula (9.7) as a colorless oil.
FAB-MS : 712 $(M+H)^+$.
^{1}H-NMR(CD_3OD):7.38 (m, 5H); 5.12 (s, 2H); 5.05 (m, 1H); 4.29-4.10 (m, 4H); 3.75 (s, 3H); 3.71-3.30 (m, 5H); 2.12 (m, 2H); 1.50 (s, 3H); 1.42 (s, 9H); 1.20 (s, 9H); 1.00 (t, 2H); 0.50 (s, 9H).



(9.7)

Example 7.14: Removal of the Z-protecting group

0.855 g of (9.7) is hydrogenated over 0.300 g of Pd/C(10%) in 30 ml of tetrahydrofurane. The catalyst is removed by filtration and the filtrate is evaporated to give the pure product as a colorless oil.

FAB-MS : 578 $(M+H)^+$.

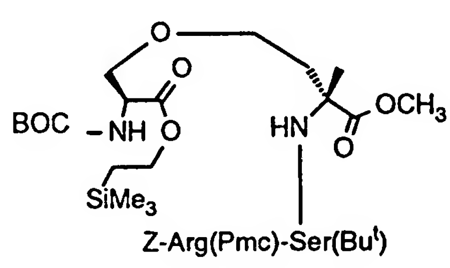
^{1}H-NMR(CD_3OD): 4.25 (m, 4H); 3.75 (s, 3H); 3.71-3.30 (m, 6H); 2.12 (m, 2H); 1.52 (s, 2H); 1.49 (s, 3H); 1.44 (s, 9H); 1.21 (s, 9H); 1.00 (t, 2H); 0.50 (s, 9H).

Example 7.15: Condensation with protected arginine

To a solution of 0.825 g of Z-Arg(Pmc)-OH in 15 ml of tetrahydrofurane are added at 0°C 0.360 g of benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium-hexafluoro-phosphate (BOP) and 0.20 ml of Et_3N. The reaction mixture is stirred for 10 min and then 0.75 g of the compound according to example 7.14 is added. The ice-bath is removed and the reaction mixture is stirred for 2.5 hours at room temperature. The reaction mixture is then poured into saturated NH_4Cl-solution and the aq. layer is three times extracted with ether. The combined organic extracts are washed with H_2O and brine, dried over sodium sulfate and the solvent is evaporated. The residue is purified by flash-chromatography (10:0.3, ether:methanol) to give the pure product of formula (9.8) as a slightly yellow foam.

FAB-MS : 1134 $(M+H)^+$.

^{1}H-NMR(CD_3OD):7.32 (m, 5H); 5.10 (s, 2H); 4.35 (m, 1H); 4.20 (m, 4H); 3.72 (s, 3H); 3.69 (m, 2H); 3.58 (m, 2H); 3.45 (m, 2H); 3.19 (t, 2H); 2.68 (t, 2H); 2.56 (s, 3H); 2.55 (s, 3H); 2.40-2.10 (m, 2H); 2.08 (s, 3H); 1.82 (t, 2H); 1.68-1.40 (m, 4H); 1.50 (s, 3H); 1.42 (s, 9H); 1.39 (s, 6H); 1.20 (s, 9H); 1.00 (t,2H); 0.50 (s, 9H).



(9.8)

Example 7.16: Removal of the 2-trimethylsilyl ethyl group

To a solution of 0.700 g of 9.8 in 20ml of tetrahydrofurane is added 0.39 g of tetrabutylammoniumfluoride. The reaction mixture is stirred for 5 hours at room temperature and is then poured into saturated NH_4Cl-solution. The aq. layer is three times extracted with ethyl acetate. The combined organic extracts are washed with H_2O and brine and the solvent is evaporated in vacuo. Purification of the residue by flash-chromatography (10:0.2, methylene chloride:methanol) gives the pure product as a slightly yellow foam.
FAB-MS : 1034 $(M+H)^+$.
^{1}H-NMR(DMSO):8.40 (d, 1H); 7.82 (m, 1H); 7.75 (d, 1H); 7.62 (d, 1H); 7.32 (m, 5H); 6.02 (d, 1H); 5.00 (s, 2H); 4.35 (m, 1H); 4.05 (m, 1H); 3.79 (m, 1H); 3.66 (s, 3H); 3.42-3.23 (m, 5H); 3.19 (m, 2H); 3.00 (m, 3H); 2.60 (t, 2H); 2.49 (s, 3H); 2.48 (s, 3H); 2.10 (s, 3H); 1.82-1.40 (m, 4H); 1.75 (t, 2H); 1.42 (s, 9H); 1.40 (s, 3H); 1.22 (s, 6H); 1.10 (s, 9H).

Example 7.17: Removal of the Z-protecting group

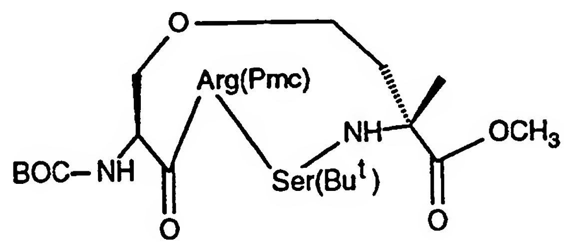
0.555 g of the product according to example 7.16 is hydrogenated over 0.110 g of Pd/C (10%) in 15 ml of methanol. The catalyst is removed by filtration and the filtrate is evaporated to give the pure product as a white powder.
FAB-MS : 900 $(M+H)^+$.
^{1}H-NMR(CD_3OD):4.35 (m, 1H); 4.05 (m, 1H); 3.78-3.45 (m, 7H); 3.65 (s, 3H); 3.20 (m, 3H); 2.68 (t, 2H); 2.58 (s, 3H); 2.56 (s, 3H); 2.12 (m, 1H); 2.10 (s, 3H); 1.82 (t, 2H); 1.70-1.40 (m, 4H); 1.50 (s, 3H); 1.42 (s, 9H); 1.30 (s, 6H); 1.20 (s, 9H).

Example 7.18: Cyclization

To a solution of 0.207 g of 1-hydroxybenzotriazole and 0.280 g of dicyclohexylcarbodiimide in 30 ml of dimethylformamide is added dropwise within 9.5 hours a solution of 0.122 g of compound according to example 7.17 dissolved in 30 ml of dimethylformamide. The reaction mixture is stirred at room temperature for 15 hours, then the solvent is evaporated in vacuo and the residue is purified by flash-chromatography (10:0.3, methylene chloride : methanol) to give the pure product of formula (9.9) as a slightly yellow powder.

FAB-MS : 882 $(M+H)^+$.

^{1}H-NMR(CD_3OD):4.41 (m, 1H); 4.22 (m, 1H); 4.02 (m, 1H); 3.82 (m, 1H); 3.72-3.48 (m, 4H); 3.69 (s, 3H); 3.50 (m, 1H); 3.2 (t, 2H); 2.69 (t,2H); 2.57 (s, 3H); 2.56 (s, 3H); 2.10 (s, 3H); 2.02 (m, 2H); 1.92-1.75 (m, 2H); 1.84 (t, 2H); 1.65-1.58 (m, 2H); 1.51 (s, 3H); 1.43 (s, 9H); 1.30 (s, 6H); 1.18 (s, 9H).



(9.9)

Example 7.19: Cleavage of the methyl ester

To a solution of 0.063 g of (9.9) in 1.4 ml of methanol is added a solution of 0.008 g of LiOH in 0.4 ml of H_2O. The reaction mixture is stirred at 45-50°C for 7 hours, then diluted with H_2O and the aq. layer is once extracted with ether. The aq. layer is acidified with 1% $KHSO_4$-solution and again extracted with ethyl acetate. The combined ethyl acetate extracts are washed with brine and dried over sodium sulfate. Evaporation of the solvent yields the pure product as a white powder.

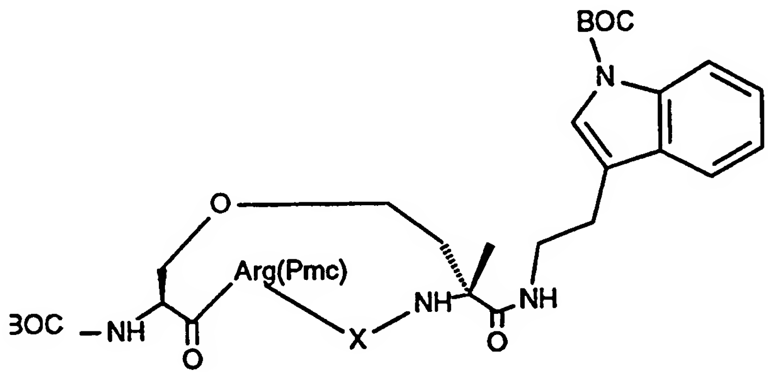
FAB-MS : 868 $(M+H)^+$.

^{1}H-NMR(CD_3OD): 4.41 (m, 1H); 4.22 (m, 1H); 4.00 (m,1H); 3.82 (m, 1H); 3.72-3.48 (m, 5H); 3.20 (t, 2H); 2.69 (t, 2H); 2.57 (s, 3H); 2.56 (s, 3H); 2.10 (s, 3H); 2.02 (m, 2H); 1.92-1.75 (m, 2H); 1.84 (t, 2H); 1.65-1.57 (m, 2H); 1.51 (s, 3H); 1.43 (s, 9H); 1.30 (s, 6H); 1.18 (s, 9H).

Example 7.20: Condensation with 2-aminoethyl indole

To a solution of 0.055 g of the compound according to example 7.19 in 2 ml of methylene chloride are added 0.014 g of dicyclohexylcarbodiimide and 0.011 g of 1-hydroxybenzo-triazole. The reaction mixture is stirred for 10 min. at room temperature then a solution of N-Boc-3-(2-aminoethyl)indole in 2 ml of methylene chloride, is added. The reaction mixture is stirred for 4 hours at room temperature and then the solvent is evaporated in vacuo. The residue is purified by flash-chromatography (10:0.5, methylene

chloride:methanol) to give a 1:1 mixture of two diastereomeric products of formula (9.10).
FAB-MS : 1110 $(M+H)^+$.
^1H-NMR(CD_3OD): 7.60 (d, 1H); 7.49 (d, 1H); 7.40 (s, 1H); 7.30-7.15 (m, 2H); 4.49 (m, 1H); 4.30 (t, 1H); 4.20 (m, 1H); 4.10 (m, 1H); 3.80-3.70 (dd, 1H); 3.70-3.50 (m, 4H); 3.50 (t, 2H); 3.20 (dd, 1H); 2.92 (q, 2H); 2.82-2.60 (m, 2H); 2.52 (s, 3H); 2.50 (s, 3H);2.10 (s, 3H); 2.00-1.50 (m, 8H); 1.50 (d, 3H); 1.40 (s, 9H); 1.35 (s, 6H); 1.15 (d, 9H).

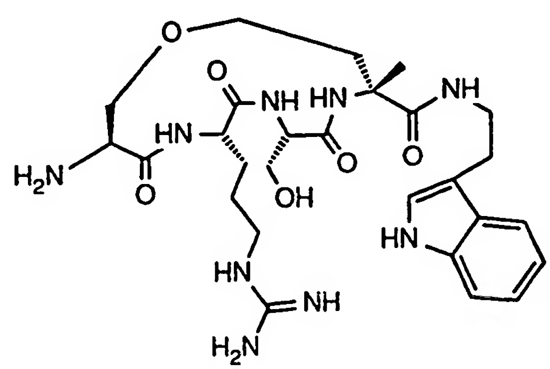


(9.10)

X= (L)-Ser(Bu^t) or (D)-Ser(Bu^t)

Example 7.21: Removal of the protecting-groups and separation of the diastereoisomeres

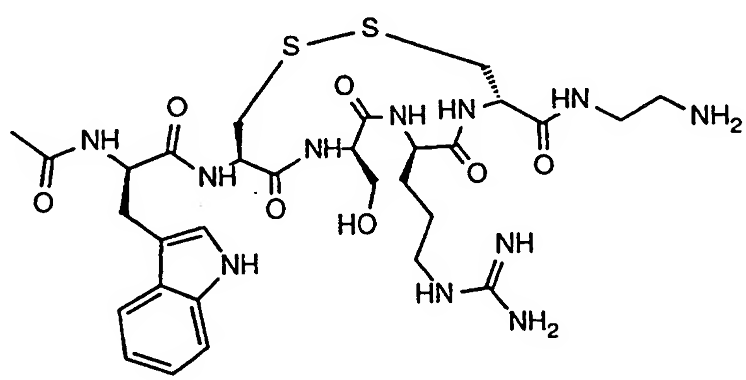
0.030 g of (9.10) is dissolved in 1 ml of trifluoroacetic acid/ethanedithiole (85:15) and is stirred for 4.5 hours at room temperature. Then the solvent is evaporated in vacuo and the residue is solidified with ether. Separation of the two diastereomeric products is achieved by HPLC, using a Nucleosil reversed-phase C_{18} column; gradient: 10% CH_3CN (0.09% trifluoroacetic acid) / 90% H_2O (0.09% trifluoroacetic acid) to 20% CH_3CN (0.09% trifluoroacetic acid) / 80% H_2O (0.09% trifluoroacetic acid) within 50 min.



(9)

R_t (compound of formula 9) = 30.3 min; FAB-MS : 588 $(M+H)^+$
R_t ((D)-isomer) = 29.1 min; FAB-MS : 588 $(M+H)^+$.
^1H-NMR(D_2O) of compound of formula (9) : 7.62 (d, 1H); 7.42 (d, 1H); 7.20 (t, 1H); 7.18 (s, 1H); 7.10 (t, 1H); 4.90 (m, 1H) 4.43 (t, 1H); 4.18 (s, 1H); 4.16 (t, 1H); 3.85 (dd, 1H); 3.67 (m, 2H); 3.58 (t, 2H); 3.31 (dd, 1H); 3.19 (m, 2H); 3.10 (m, 1H); 2.98 (t, 2H); 2.90 (t, 2H); 1.90-1.57 (m, 6H); 1.42 (s, 3H).
^1H-NMR(D_2O) of (D)-isomer :7.63 (d, 1H); 7.42 (d, 1H); 7.20 (t and s, 2H); 7.10 (t, 1H); 4.90 (m, 1H); 4.50 (m, 1H); 4.10 (t, 1H); 4.05 (m, 1H); 3.79 (m, 2H); 3.60 (m, 1H); 3.50 (m, 4H); 3.29 (m, 1H); 3.19 (m, 2H); 3.00 (m, 3H); 1.84-1.42 (m, 6H); 1.40 (s, 3H).

Example 8: Synthesis of a compound of the formula (10)



(10)

Example 8.1: Fmoc-D-Cys(Trt)-TAB-ester-resin

Fmoc-D-Cys(Trt) (0.24 mmol) are dissolved in 0.4 ml DMA (dest.), 2 ml DCE and 0.40 g TAB-ester-resin (0.24 mmol) (Novabiochem) are added under slow stirring. The mixture is cooled to 0-5°C and a solution of 0.1 g DCCI (0.48 mmol) in 0.2 ml DMA and 0.06 ml DCE is added. After 5 minutes at 0-5°C a solution of 5.9 mg DMAP (0.048 mmol) in 0.4 ml DCE is added. After 20 minutes at 0-5°C 27.3 µl N-methylmorpholine is added. The reaction mixture is then shaken for 4 hours. The resin is washed as follows: 6 times with DMA (6 ml each), 5 times with isopropanole (6 ml each). The resin is dried under reduced pressure: 0.5 g (with traces of solvents).

Example 8.2: Synthesis of the protected resin-bound peptide
Ac-D-Trp-Cys(Trt)-D-Ser(But)-D-Arg(Pmc)-D-Cys(Trt)-(TAB-ester-resin)

Starting from 0.25 g Fmoc-D-Cys(Trt)-TAB-ester-resin (0.15 mmol), the following process steps, repeated for each step, are carried out:

- single wash for 0.8 minutes with isopropanol;

- pre-activation for first coupling: 1.4 mMole of the respective appropriately protected Fmoc-amino acid are dissolved in 3.08 ml of 0.5 M HOBt in DMA and 0.77 ml of a 2 M solution of DICD in DMA are added. The reaction mixture is maintained for about 40 minutes, and then used in that form. During this time the following washings and the removal of the Fmoc protecting group is already proceeding;

- eight treatments of the resin starting material, each of 1 minute's duration, with a 20 % solution of piperidine in DMA (removal of the Fmoc protecting group);

- three washes, each of 0.4 minute's duration, with DMA (previously degassed under reduced pressure);

- single wash for 0.8 minutes with isopropanole;

- five washes, each of 0.4 minute's duration, with DMA (degassed);

- addition of the coupling reagent, which has been prepared in the meantime (see above). The reaction mixture is maintained with shaking for about 60 minutes. Twice, 5 minutes and 15 minutes after beginning of the coupling, 120 µl DIPEA (0.7 mMole) are added.

- removal of the coupling mixture after 60 minutes;

- two washes for 0.4 minutes with DMA (degassed);

- single treatment for 5 minutes with approximately 15 ml of a 1:1:8 mixture (v/v/v) of acetic anhydride, pyridine and DMA (for the acetylation of amino groups that are still free in the growing peptide chain);

- four washes, each of 0.4 minute's duration, with DMA (degassed);

- two washes, each of 0.8 minute's duration, with isopropanol.

In this manner there is obtained the following Fmoc-peptide/resin intermediate:

Ac-D-Trp-Cys(Trt)-D-Ser(But)-D-Arg(Pmc)-D-Cys(Trt)-(TAB-ester-resin) by sequential coupling of Fmoc-D-Arg(Pmc), Fmoc-D-Ser(But), Fmoc-Cys(Trt) and Fmoc-D-Trp.

At the end of the synthesis the Fmoc-group is removed and the terminal amino group is acetylated with the capping agent as described above. After final washings with isopropanole, the resin is dried under reduced pressure: 0.38 g.

Example 8.3: Cleavage of the linear protected peptide from the resin

0.38 g peptide loaded resin (0.13 mmol) is shaken with 100 ml of a mixture of 99 % AcOH and DCM (1:9 v/v) for 1.5 hours. The resin is filtered and washed twice with DCE (80 ml each), once with a mixture of DCE and TFE (1:1 v/v) (80 ml) and four times with TFE (80 ml each). The combined filtrates are concentrated under reduced pressure at 25-30°C and the oily residue is dissolved in ca. 200 ml DMSO (distilled) and lyophilized. For complete removal of traces of AcOH which would interfere with the following process of cyclization, two additional lyophilizations are applied to the product (from 50 ml DMSO each). After lyophilization from DMSO a slightly yellow powder is obtained: 0.21 g.

Tlc, CM. single spot R_f ca. 0.4.
MALDI, positive mode, 1606.7 (calculated: 1604.1).

Example 8.4: Coupling of the linear protected peptide to mono-Boc-1,2-diamino-ethane

0.101 g of the linear protected peptide (65 µmol) is dissolved in 0.70 ml DMA (degassed), 12.2 µl DIPEA (71.5 µmol) and 28.8 mg TBTU (65 µmol) are added and preactivation is performed for 3 minutes. 41.9 mg mono-Boc-1,2-diamino-ethane (262 µmol) is added and the mixture is kept for 16 hours. The crude product is precipitated with 10 ml water

(0-5°C), filtrated and washed with 3 ml water. The solid is dried over P_2O_5 under reduced pressure: 95 mg.
Tlc CM, R_f ca. 0.85.
MALDI, positive mode, 1746.5 (calculated: 1746.0).

Example 8.5: Cleavage of protecting groups

In order to remove the polymeric carrier, and the acid-labile protecting groups, 95 mg of the synthesized peptide are shaken twice for 5 minutes with 4 ml of a 85:15 mixture (v/v) of TFA (95 %) and ethanedithiole and then filtered. The filtration residue is then washed three times with 4 ml each of DCE and three times with 4 ml each of TFE. The combined filtrates and washing liquids are concentrated under reduced pressure to a volume of about 2 ml, and the crude peptide is precipitated by the addition of 15 ml of a 1:1 mixture (v/v) of DIPE and petroleum ether (low-boiling). The precipitate is isolated by filtration, washed with 5 ml of the precipitation mixture and dried under reduced pressure.

For completely removing the protecting groups, the solid is dissolved in 4 ml of the above mentioned cleavage mixture and kept for two hours, precipitated and dried under reduced pressure: 43 mg white powder.

Analytical HPLC: retention time ca. 14.3 minutes.
MALDI, positive mode 737.3 (calculated: 738.9).

Example 8.6: Cyclization of the linear peptide

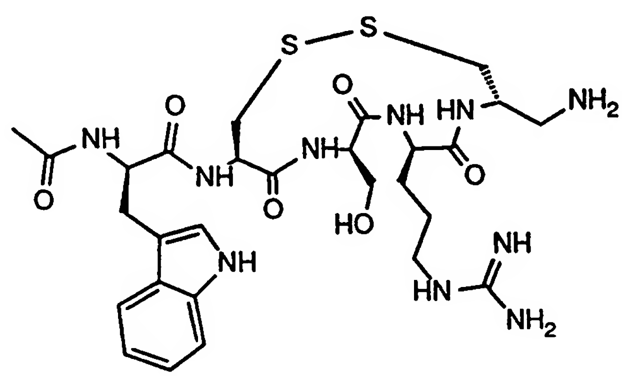
43 mg (58.4 µmol) of the peptide is dissolved in 600 ml water and the pH adjusted to 8 by addition of ammonia (10 % in water). The cyclization by disulfide formation is performed by bubbling air through the solution for 4 hours. After acidification with 2.5 ml AcOH, the solution is concentrated under reduced pressure to about 10 ml and lyophilized.

For purification, the crude peptide is dissolved in a mixture of 4.5 ml CH_3CN/water (1:9 v/v) and 0.1 ml AcOH and subjected to high pressure liquid chromatography (HPLC) under the following conditions: the column, measuring 20x250 mm, Nucleosil $7C_{18}$ (10 nm) manufactured by Machery-Nagel, Dueren, Germany; 0.1 % TFA is used as eluent

(A) and a 0.1 % solution of TFA in CH_3CN is used as eluent (B). The linear gradient is 10 % B to 90 % B in 30 minutes, the throughput speed 18 ml/min and the detection is at 215 nm wavelength. The main fraction, with a retention time of approximately 16 minutes, is collected concentrated under reduced pressure and filtrated through an ion-exchange column (15 ml), AG1-X8 (Bio-Rad), preloaded with AcOH, for exchanging TFA with AcOH. The column is extensively washed with water and the combined fractions (40 ml) are lyophilized. The compound with the formula (10) is obtained as a colorless, white powder.

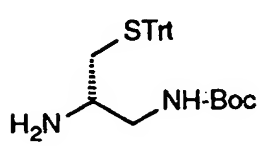
Analytical HPLC, single peak with a retention time ca. 15.3 minutes.
MALDI: positive mode 736.9 (calculated: 736.9).

Example 9: Synthesis of a compound of the formula (11)



(11)

Example 9.1: Synthesis of the compound of the formula (11.1)



(11.1)

Example 9.1.1: Reduction of the carboxyl group of Fmoc-Cys(Trt)

To a cold solution (-15°C) of 10 mmol Fmoc-Cys(Trt) in 10 ml DME, were successively added 1.11 ml N-methyl morpholine (10 mmol) and 1.36 ml isobutyl chloroformate (10 mmol). After 1 min., the precipitated N-methyl morpholine hydrochloride was removed by filtration, washed with DME (5x2 ml) and the filtrate and washings were combined in a large flask in an ice-bath. A solution of 570 mg sodium borohydride

(15 mmol) in 5 ml water was added at once, producing a strong evolution of gas, followed by 250 ml water immediately afterwards. The resulting alcohol was extracted with diethylether and purified by flash chromatography (ether/petrolether 6:4).

^{1}H-NMR ($CDCl_3$): 7.8-7.0 (m, 23 H); 4.84 (d, 1H); 4.40 (d, 2H); 4.20 (t, 1H); 3.50 (m, 4H); 2.45 (m, 1H).

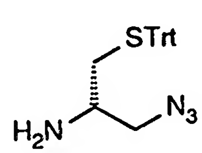
Example 9.1.2: Tosylation of the alcohol

To a solution of 9.1 g of the isolated alcohol compound according to example 9.1.1 in 20 ml of CH_2Cl_2 are added 0.65 ml of $(CH_3CH_2)_3N$, 0.710 g of toluene-4-sulfonyl chloride and 0.04 g of dimethylaminopyridine at 0°C. The ice-bath is immediately removed and the reaction mixture stirred for 4 hours at room temperature. The reaction mixture is poured on ice and extracted with methylene chloride in two portions. The combined organic extracts are dried over sodium sulfate and concentrated in vacuo. The residue is purified by flash chromatography (ether/hexane 1:1) to yield the corresponding tosylate as a white foam.

^{1}H-NMR($CDCl_3$): 7.8-7.18 (m, 27H); 4.65 (d, 1H); 4.43 (m, 2H); 4.15 (t, 1H); 3.91 (m, 2H); 3.52 (m, 1H); 2.42 (d, 2H); 1.58 (s, 3H).

Example 9.1.3: Substitution of the alcohol group with an azido group and removal of the Fmoc protecting group

To a solution of 1.5 g of the compound according to example 9.1.2 in 20 ml of dimethylformamide is added 0.160 g of sodium azide. The reaction mixture is stirred at 60°C for 3 hours and then partitioned between saturated ammonium chloride solution and ethyl acetate. The organic layer is separated, dried over sodium sulfate and concentrated in vacuo to leave a yellow oil. Flash chromatography (ether) yields the compound of the formula (11.2) as pure product.



(11.2)

^{1}H-NMR ($CDCl_3$): 7.35 (s, 15H); 3.15 (d, 2H); 2.52 (m, 1H); 2.28 (d, 2H); 1.48 (s, 2H).
IR (CH_2Cl_2): 2106 cm^{-1} (s, N_3); 1720 cm^{-1} (s, C=O).

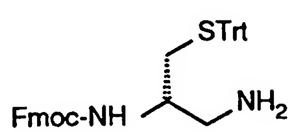
Example 9.1.4: Introduction of a Fmoc protecting group

To a solution of 1.10 g of the compound of the formula (11.2) in 20 ml of methylene chloride are added 0.5 ml of N-ethyldiisopropylamine and 0.760 g of Fmoc-Cl at 0°. The reaction mixture is warmed to room temperature and stirred for 4 hours. It is then partitioned between methylene chloride and saturated ammonium chloride solution. The organic layer is separated, dried over calcium chloride and the solvent is removed in vacuo. Purification of the crude product by flash chromatography (ether/hexane 80:20) yields the Fmoc-protected compound as a white foam.

^{1}H-NMR ($CDCl_3$): 7.80-7.20 (m, 23H); 4.40 (d, 2H); 4.20 (t, 1H); 3.54 (m, 1H); 3.30 (m, 2H); 2.41 (m, 2H).
IR (CH_2Cl_2): 2108 cm^{-1} (s, N_3); 1730 cm^{-1} (s, C=O).

Example 9.1.5: Reduction of the azido group to the amino group

To a solution of 1.5 g of the Fmoc-protected compound in 30 ml of THF is added 0.735 g of triphenylphosphine and 0.54 ml of H_2O. The reaction mixture is stirred for 12 hours at room temperature, is then poured on ice/saturated ammonium chloride solution and twice extracted with ethyl acetate. The combined organic extracts are dried over sodium sulfate and concentrated in vacuo to give the compound of the formula (11.3).



(11.3)

^{1}H-NMR ($CDCl_3$): 7.80-7.20 (m, 23H); 4.85 (d, 1H); 4.40 (d, 2H); 4.28 (t, 1H); 3.45 (dd, 1H); 2.65 (m, 2H); 2.41 (m, 2H); 1.60 (s, 2H).

Example 9.1.6: Introduction of a Boc protecting group

To a solution of 1.5 g of the compound of the formula (11.3) in 30 ml of THF are added 0.660 g of di-tert-butyl dicarbonate and 0.45 ml of N-ethyldiisopropylamine at 0°C. The ice-bath is immediately removed and the reaction mixture stirred for 2 hours at room temperature. The reaction mixture is partitioned between saturated ammonium chloride solution and ethyl acetate, the organic layer is separated, dried over sodium sulfate and concentrated in vacuo. The residue is purified by flash chromatography (ethyl acetate/hexane 2:6) to yield the pure product as a colorless oil.

^{1}H-NMR ($CDCl_3$):7.80-7.20 (m, 23H); 5.22 (d, 1H); 4.45 (d, 1H); 4.35 (d, 2H); 4.18 (t, 1H); 3.58 (m, 1H); 3.10 (t, 2H); 2.40 (t, 2H); 1.40 (s, 9H).

Example 9.1.7: Removal of the Fmoc protecting group

To 15 ml of dimethylamine in dimethylformamide is added 2 g of the compound according to example 9.1.6. The reaction mixture is stirred at room temperature for 30 min and then is partitioned between saturated ammonium chloride solution and ethyl acetate, the organic layer is separated, dried over sodium sulfate and concentrated in vacuo. The residue is purified by flash chromatography (ethyl acetate) to yield the pure compound of the formula (11.1).

^{1}H-NMR (CD_3OD): 7.46-7.18 (m, 15H); 2.90 (dd, 1H); 2.82 (dd, 1H); 2.48 (m, 1H); 2.35 (dd, 1H); 2.22 (dd, 1H); 1.40 (s, 9H).

Example 9.2: Fmoc-D-Arg(Pmc)-(TAB-ester-resin)

Fmoc-Arg(Pmc) is attached to the resin in an analogue manner as described in example 8.1.

Example 9.3: Ac-D-Trp(Boc)-Cys(Trt)-D-Ser(But)-D-Arg(Pmc)-(TAB-ester-resin)

0.30 g Fmoc-D-Arg(Pmc)-TAB-ester-resin (0.19 mmol) is subjected to a solid-phase

synthesis analogues to process example 8.2, attaching in a sequential manner the following amino acid derivatives: Fmoc-D-Ser(But), Fmoc-Cys(Trt) and Fmoc-D-Trp. At the end of the synthesis the Fmoc-group is removed and the terminal amino group is acetylated with the capping agent described in example 8.2. After final washings with isopropanole, the resin is dried under reduced pressure: 0.47 g.

Example 9.4: Cleavage of the linear protected peptide from the resin

0.39 g peptide loaded resin (0.17 mmol) are treated as described in example 8.3. After lyophilization from DMSO a slightly yellow powder is obtained: 0.19 g.

Tlc, CM. single spot R_f ca. 0.35.
MALDI, positive mode, 1258.1 (calculated: 1258.6).

Example 9.5: Coupling of the linear protected peptide to compound of the formula (11.1)

69 mg of the linear protected peptide (55 µmol) is dissolved in 0.40 ml DMA (degassed), 10.2 µl. DIPEA (60.5 µmol) and 24.4 mg TBTU (55 µmol) are added and preactivation is performed for 3 minutes. 49.3 mg of the compound of the formula (11.1) (110 µmol) is added and the mixture is kept for 16 hours. The crude product is precipitated with 3.0 ml water (0-5°C), filtrated and washed with 1.0 ml water. The solid is dried over P_2O_5 under reduced pressure: 85 mg.
Tlc CM, R_f ca. 0.91.
MALDI, positive mode, 1688.7 (calculated: 1689.2).

Example 9.6.: Cleavage of protecting groups

85 mg crude protected peptide are subjected to protecting group cleavage as described in example 8.5, part 'complete removal of protecting groups': 39 mg white powder, analytical HPLC: retention time ca. 13.1 minutes.

Example 9.7: Cyclization of the linear peptide

39 mg peptide (57.3 μmol) is oxidized as described in example 8.6 in 400 ml water. The crude product is subjected to the HPLC-purification and acetate conversion under standard conditions (example 8.6), but with a gradient running from 10% to 90% in 30 minutes). Retention time ca. 15 minutes. After lyophilization, the compound of the formula (11) is obtained as a white powder.

Analytical HPLC, single peak with a retention time ca. 14.8 minutes.
MALDI: positive mode 678.9 (calculated: 679.8).

Example 10: Proteolytic stability

To test the proteolytic stability of TRSAW and analogues in a physiological relevant medium ($t_{\frac{1}{2}}$), the peptides were incubated at 37°C in rat serum at a concentration of 200 μM. Aliquots of the incubation mixture (100 μl) were withdrawn at various times, mixed with 60 μl of 20 % perchloric acid to precipitate protein and centrifuged at 12000 g for 8 min. Residual starting material and degradation products remaining in the serum were determined by reversed phase HPLC using a Nucleosil C_{18} and Nucleosil C_8 column with UV monitoring at 216 nm, flow rate 1.0 ml/min and a gradient of 10-90 % CH_3CN over 30 min. (table 1).

Table 1:

Compound	$t_{\frac{1}{2}}$ [min.]
TRSAW	5
(3)	22
(5)	18
(6)	22

Example 11: Effects of TRASW-analogues on trabecular bone ovariectomized rats

Female rats of the Sprague-Dawley-derived strain Tif:RAIF (Sisseln) weighing 130-150 g body weight were used. The animals were fed ad libitum a standard rat breeding diet (NAFAG, Grossau, Switzerland) containing 1.2 % calcium, 0.9 % phosphorus and 24.8 % protein. Ovariectomy (OVX) was performed by electrotomy via the dorsal route in anesthesia with vetanarcol (Veterinaria, Zurich, Switzerland). The animals were randomly divided into groups of 8.

Treatment was started on the day of ovariectomy. TRASW or its analogues were dissolved in phosphate buffered saline containing 0.1 % rat serum albumin. The test compounds were administered by daily subcutaneous injection on 5 days per week. Intact and ovariectomized control animals received the appropriate vehicle. Treatment lasted for 3 weeks.

The animals were killed 24 hours after the last injection. Both femurs were freed of connective tissue and the amount of trabecular bone was determined according to Gunness-Hey and Hock (Metab. Bone Dis. & Rel. Res. (1984), 5, 177-181). The femurs were cut in half at the mid-diaphysis using a dental saw and the proximal halves were discarded. With a scalpel the epiphysis of the distal half was cut off and the bone was split into saggital halves. The marrow was flushed out with water. With a dental curette the trabecular bone was scraped out of both cortical shells, combined and put into 5 % TCA. After standing 16 hours at room temperature, the TCA extract was removed and used for the determination of calcium by atomic adsorption spectroscopy.

Results are given as mean ± SEM (table 2). Statistical analysis of the data was by two-tailed Student's paired test.

Table 2:

Test compound	Dose⁺ µg/ml	Calcium content of the trabecular bone of the femur [mg] Intact control	OVX control	OVX + test compound
TRSAW	0.2	4.06±0.12	2.96±0.11*	3.28±0.11
"	2			3.71±0.14#
"	20			4.45±0.22#
(3)	1	5.32±0.28	3.24±0.12*	3.45±0.14
"	4			3.68±0.13#
"	10			3.32±0.13

+: Dayly dose in µg/kg rat

#: $p < 0.05$ against OVX control

*: $p < 0.05$ against intact control

Example 12: Influence on protein kinase-C activation

Compounds capable of protein kinase-C (PKC) activation are most probably also active in anabolic actions on bone and effective drugs to reverse bone loss in osteoporotic patients (H. Jouishomme et al., Endocrinology (1992), 130, 53-60). Therefore, the PKC-activation of compound (3) is compared to Acetyl-TRSAW that is known to be inactive in osteoporosis protection and TRSAW-amide that is known to be active (Gagnon et al., J. Bone and Mineral Res. (1993), 8, 497-503). The activity is measured in % of difference between membrane and cytosol located PKC.

Example 12.1: Stimulation with the test compounds

Serum free cells (osteosarcoma cell line UMR 106) are stimulated with the compound in question (10 minutes at 30°C, concentrations see table 1)

Example 12.2: Separation of cytosolic and membrane associated PKC

The cells are washed with cold saline twice (4°C) and scraped with 800 µl of a buffer (A) consisting of:

85.60 g sucrose
0.58 g EDTA
2.42 g Tris
1.90 g EGTA
add 1000 ml H_2O and adjusted to pH 7,5 using HCl

before usage 10 ml of this buffer is supplied with

20 µl DTT (1 M)
20 µl PMSF (50 mM)
40 µl leupeptin (2.5 mg/ml)

and sonicated in a centrifuge tubes for 10 sec. at 4°C low intensity. Then the cells are centrifuged for 60 min at 100,000 g and the supernatant (cytosolic PKC) is separated. The pellet is suspended using 776 µl of buffer A; 25 µl triton (10%) is added; the tubes are shaken for 1 h at 4°C and again centrifuged for 60 min at 100,000 g. The supernatant contains the membrane associated PKC.

5 ml of DEAE sephacel are washed 3 times with 20 ml buffer (B) consisting of:

2.42 g/l Tris, pH 7.5
0.29 g/l EDTA
0.38 g/l EGTA

after each wash the resin is separated by centrifugation. Finally the resin is washed with buffer (C), consisting of:

40 ml of buffer (B)
80 µl DTT (1 M)
80 µl PMSF (50 mM)
160 µl leupeptin (2.5 mg/ml)

1650 µl of buffer (C) is added to 333 µl of washed resin. Cytosolic and the membrane associated PKC are added in separated compartments and the suspensions are mixed at 4°C for 1 h. The supernatant is removed by centrifugation and the resins are washed 3 times using 1 ml buffer (B) each and 1 time using 1 ml buffer (B) containing 20 mM NaCl.

The resin bound PKC is eluted by adding 300 µl NaCl (130 mM), shaking for 15 min. at 4°C and isolating the supernatant after centrifugation. Remaining resin bound PKC is eluted by adding 200 µl NaCl (130 mM), shaking for 15 min. at 4°C and isolating the supernatant after centrifugation. The two supernatants are combined.

Example 12.3: Measurement of the activities

Buffers:

	blank	reaction mix
Tris (0.2 M; pH 7.5)	25 µl	25 µl
Mg acetate (0.5 M)	5 µl	5 µl
leupeptin (0.5 mg/ml)	25 µl	25 µl
radioactive ATP (100 µm)	25 µl	25 µl
$CaCl_2$ (2.5 mMol)	2.5 µl	2.5 µl
phosphatidyl-serine mix	-	80 µl
Tris (0.02 M)	80 µl	-
H_2O	30.5 µl	30.5 µl
peptide (7 mM)	7 µl	7 µl

phosphatidyl-serine mix:

150 µl phosphatidylserine (10 mg/ml)
60 µl diolein (5 mg/ml)
add 5 ml tris (20 mM, pH 7.5)

peptide:

substrat for the phosphorylation (FKKSFKL-NH_2)

50 µl of the cytosol and membrane fractions are added to 200 µl of blank-mixture (reaction time 5 min. at 30°C). The reaction is stopped by addition of 125 µl of concentrated acetic acid and 100 µl of these solutions are placed on 3 by 3 cm whatman® filter paper. This experiment is repeated, using the reaction-mix instead of the blank-mix. Each experiment using the blank-mixture is carried out 2 times and each experiment using the reaction-mix is carried out 3 times.

The filter papers are placed in scintillation vials and counted. The average count for the blank-mixes is subtracted from the average for the reaction mix and the amount of protein in each sample is calculated using the specific activity of the radioactive ATP.

The differences between cytosolic and membrane associated PKC is shown in table 1:

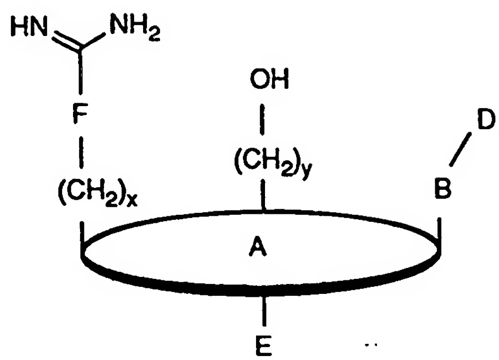
Table 1:

Conc. of test-compound in mol/l	Difference between % of PKC in cytosol and membrane		
	TRSAW-amide	Acetyl-TRSAW	compound (3)
0	0	0	0
10^{-11}	16	9	14
10^{-10}	22	2	31
10^{-9}	27	21	48
10^{-8}	42	3	69

Compound (3) shows an even increased activity compared to also active TRSAW-amide. Acetyl-TRSAW shows no characteristic activity as expected.

Claims

1. A cyclic compound of the formula (1)



(1)

wherein

A = cyclic radical having 12-17 ring forming atoms,

B = a spacer group, linked to the cyclic radical A via a carbon or nitrogen atom, having 1 to 6 carbon atoms and 0 to 2 nitrogen and 0 or 1 oxygen atoms in the backbone of the chain; wherein the carbon atoms can be substituted by oxo, hydroxy, sulfo, C_1-C_4alkyl, morpholino, amino, carboxy and/or by a radical of a naturally occurring amino acid or a radical of a peptide containing 2-5 naturally occurring amino acids; and the nitrogen atoms can be substituted by C_1-C_4alkyl, C_1-C_4alkanoyl and/or by a radical of a naturally occurring amino acid or a radical of a peptide containing 2-5 naturally occurring amino acids;

D = aryl-C_1-C_4alkyl wherein the aryl radical is mono- or bicyclic and unsubstituted or substituted with OH, SH, NH_2 or halogen;

E = NH_2; NH_2 substituted with C_1-C_4alkyl or amino-C_1-C_4alkyl; C_1-C_4alkyl substituted with one or more amino groups; a five- or six-membered one or two nitrogen containing heterocyclic radical; or NH_2 substituted by an acyl radical of a naturally occurring amino acid or an acyl radical of a peptide containing 2-5 naturally occurring amino acids;

F = NH or CH_2;

x = 1 to 6;

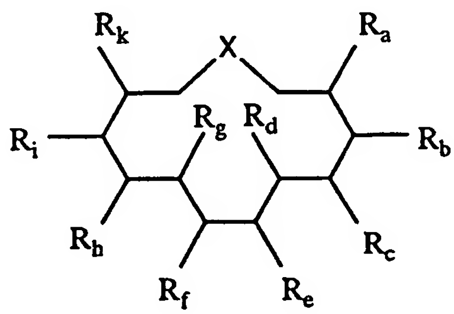
y = 0 to 4;

with the proviso that the residues bearing the $(CH_2)_x$-F-C(=NH)NH_2, $(CH_2)_y$-OH and B-D radicals are located above the plane assumed by the cyclic radical A and the radical E is located below the plane assumed by the cyclic radical A;

or a pharmacologically acceptable salt thereof.

2. A cyclic compound according to claim 1, wherein the distance between the binding sites of the B-D-group to the cyclic radical and the E-group to the cyclic radical is 0.50 to 0.65 nm, the distance between the binding sites of the $(CH_2)_y$-group to the cyclic radical and the E-group to the cyclic radical is 0.50 to 0.62 nm, and the distance between the binding sites of the $(CH_2)_x$-group to the cyclic radical and the B-D-group to the cyclic radical is 0.55 to 0.75 nm.

3. A cyclic compound according to claim 1 of formula (1a)



(1a)

wherein up to 6 of the ring forming C-atoms can be replaced by oxygen, sulfur or nitrogen; and the ring forming atoms can additionally be substituted by up to 6 C_1-C_4alkyl, oxo, hydroxy, sulfo and amino radicals; and

X = C_1-C_4alkylen or C_1-C_4alkenylene wherein up to 2 C-atoms can be replaced by oxygen, sulfur, disulfide, sulfoxy, sulfonyl or nitrogene and wherein the C_1-C_4alkylene or alkenylene group can be substituted by methyl, ethyl, oxo or amino;

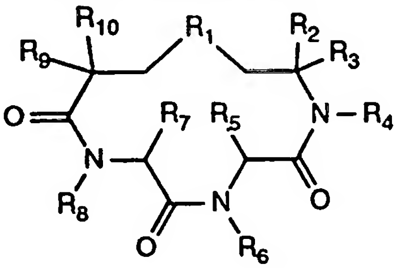
R_a or R_b = radical of a naturally occurring amino acid or a radical of a peptide containing 2-5 naturally occurring amino acids, wherein the first or second amino acid after the ring forming atom is an amino acid carrying an aromatic side chain; or C_1-C_7alkyl-aryl, wherein the C-atoms of the C_1-C_7alkyl spacer group can be replaced by up to 3 nitrogen or oxygen; and wherein the spacer group can be substituted by oxo; C_1-C_4alkyl, amino and carboxy;

R_c, R_d or R_e = hydroxy or C_1-C_4alkyl substituted with hydroxy;

R_f, R_g or R_h = C_2-C_5alkyl substituted with -C(=NH)NH_2 or -NH-C(=NH)NH_2; preferred -CH_2-CH_2-CH_2-NH-C(=NH)NH_2;

R_i or R_k = NH_2; NH_2 substituted with C_1-C_4alkyl or amino-C_1-C_4alkyl; C_1-C_4alkyl substituted with one or more, amino groups; a five- or six-membered one or two nitrogen containing heterocyclic radical; or a radical of a naturally occurring amino acid or of a peptide containing 2-5 naturally occurring amino acids;

4. A heterocyclic according to claim 1 of the formula (2)



(2)

wherein

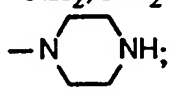
R_1 = CH_2, C_2H_4, C_2H_2, $CH(CH_3)$-CH_2, S, CH_2-S, CH_2-S-S, S-S-CH_2, S-S, S=O, O=S=O, NH, NH-CO, O, Se; CH_2, C_2H_4, C_2H_2, $CH(CH_3)$-CH_2, S, CH_2-S, CH_2-S-S, S-S, S=O, O=S=O, NH, NH-CO, O, O-CH_2, CH_2-O, or Se;

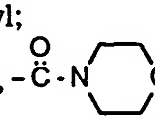
R_2 = -C_1-C_6alkyl-aryl or -CO-NH-CH(C_1-C_3alkyl-aryl)R_{11};

R_3, R_4, R_6 and R_9 independently of each other are hydrogen or C_1-C_4alkyl;

R_5 = hydroxy or C_1-C_4alkyl substituted with hydroxy;

one of the residues R_7 and R_8 is C_2-C_5alkyl substituted with -C(=NH)NH_2 or -NH-C(=NH)NH_2; and the other residues is hydrogen or C_1-C_4alkyl;

R_{10} = NH_2; NH_2 substituted with C_1-C_4alkyl; C_1-C_4alkyl substituted with NH_2; ; -NH-CO-CH_2-NH_2 or -NH-CH_2-CH_2-NH_2 unsubstituted or substituted with methyl or ethyl;

R_{11} = hydrogen, -COOH, -$CONH_2$,  or -CO-R_{12};

R_{12} = a radical of a naturally occurring amino acid or a radical of a peptide containing 2-5 naturally occurring amino acids;

5. A heterocyclic compound according to claim 4 wherein

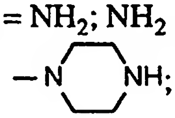
R_1 = CH_2, C_2H_4, S, CH_2-S, CH_2-S-S, S-S, NH, NH-CO, O, O-CH_2, CH_2-O, Se;

R_2 = -C_1-C_6alkyl-R_{13} or -CO-NH-CH(C_1-C_3alkyl-R_{13})R_{11};

R_3, R_4, R_6 and R_9 independently of each other are hydrogen, methyl or ethyl;

R_5 = hydroxy or C_1-C_3alkyl substituted with hydroxy;

one of the residues R_7 and R_8 is C_2-C_5alkyl substituted with -C(=NH)NH_2 or -NH-C(=NH)NH_2; and the other residue is hydrogen, methyl or ethyl;

R_{10} = NH_2; NH_2 substituted with C_1-C_4alkyl; C_1-C_4alkyl substituted with NH_2; ; -NH-CO-CH_2-NH_2; -NH-CO-CH(CH_3)-NH_2 or -NH-CH_2-CH_2-NH_2;

R_{11} = hydrogen, -COOH, -$CONH_2$ or -CO-R_{12};

R_{12} = a radical of a naturally occurring amino acid;

R_{13} = phenyl, hydroxyphenyl, naphthyl, indolyl, pyridyl, quinolyl, isoquinolyl or pyrrolyl.

6. A heterocyclic compound according to claim 4 wherein

R_1 = CH_2, CH_2-CH_2, S, CH_2-S, CH_2-S-S, S-S, NH-CO, O, O-CH_2, CH_2-O;

R_2 = -C_3-C_6alkyl-R_{13} or -CO-NH-CH(C_1-C_3alkyl-R_{13})R_{11};

R_3, R_4, R_6 and R_9 independently of each other are hydrogen, methyl or ethyl;

R_5 = hydroxy, hydroxymethyl or hydroxyethyl;

one of the residues R_7 and R_8 is C_2-C_5alkyl substituted with -C(=NH)NH_2 or -NH-C(=NH)NH_2; and the other residue is hydrogen, methyl or ethyl;

R_{10} = NH_2; NH_2 substituted with C_1-C_2alkyl; C_1-C_2alkyl substituted with NH_2; -NH-CO-CH_2-NH_2 or -NH-CO-CH(CH_3)-NH_2;

R_{11} = hydrogen, -COOH, -$CONH_2$ or -CO-R_{12};

R_{12} = a radical of a naturally occurring amino acid;

R_{13} = phenyl, hydroxyphenyl or indolyl.

7. A heterocyclic compound according to claim 4 wherein

R_1 = CH_2, CH_2-CH_2, S, CH_2-S, CH_2-S-S, S-S, NH-CO, O, O-CH_2;

R_2 = -C_3-C_6alkyl-R_{13} or -CO-NH-CH(C_1-C_3alkyl-R_{13})R_{11};

R_3, R_4, R_6 and R_9 independently of each other are hydrogen or methyl;

R_5 = hydroxy, hydroxymethyl or hydroxyethyl;

one of the residues R_7 and R_8 is C_2-C_5alkyl substituted with -C(=NH)NH_2 or -NH-C(=NH)NH_2; and the other residue is hydrogen, methyl or ethyl;

R_{10} = NH_2; -NH-CH_3, -NH-CH_2-CH_3; -CH_2-NH_2; -CH_2-CH_2-NH_2; -NH-CO-CH_2-NH_2 or -NH-CO-CH(CH_3)-NH_2;

R_{11} = hydrogen, -COOH, -$CONH_2$ or -CO-R_{12};

R_{12} = a radical of a naturally occurring amino acid;

R_{13} = phenyl, hydroxyphenyl or indolyl.

8. A heterocyclic compound according to claim 4 wherein

R_1 = CH_2, CH_2-CH_2, S, CH_2-S, CH_2-S-S, S-S, NH-CO, O-CH_2;

R_2 = -CO-NH-CH(C_1-C_3alkyl-R_{13})R_{11};

R_3 = hydrogen or methyl;

R_4, R_6 and R_9 are hydrogen;

R_5 = hydroxy, hydroxymethyl or hydroxyethyl;

one of the residues R_7 and R_8 is CH_2-CH_2-CH_2-NH-C(=NH)NH_2; and the other residue is hydrogen;

R_{10} = NH_2; -NH-CH_3; -CH_2-NH_2; -CH_2-CH_2-NH_2; -NH-CO-CH_2-NH_2 or -NH-CO-CH(CH_3)-NH_2;

R_{11} = hydrogen, -COOH or -$CONH_2$;

R_{13} = phenyl, hydroxyphenyl or indolyl.

9. A heterocyclic compound according to claim 4 wherein

R_1 = CH_2, CH_2-CH_2, S, CH_2-S, CH_2-S-S, S-S, NH-CO, O-CH_2;

R_2 = -CO-NH-CH(CH_2-R_{13})R_{11};

R_3 = hydrogen or methyl;

R_4, R_6 and R_9 are hydrogen;

R_5 = hydroxy or hydroxymethyl;

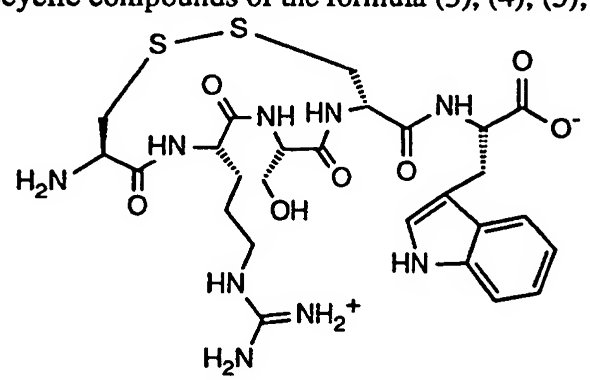
one of the residues R_7 and R_8 is -CH_2-CH_2-CH_2-NH-C(=NH)NH_2; and the other residue is hydrogen;

R_{10} = NH_2; -NH-CO-CH_2-NH_2 or -NH-CO-CH(CH_3)-NH_2;

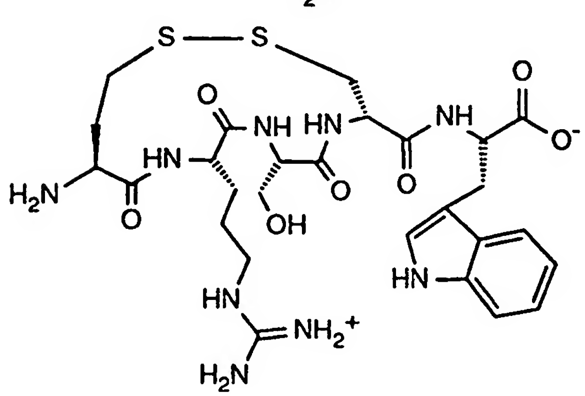
R_{11} = hydrogen, -COOH or -$CONH_2$;

R_{13} = indolyl.

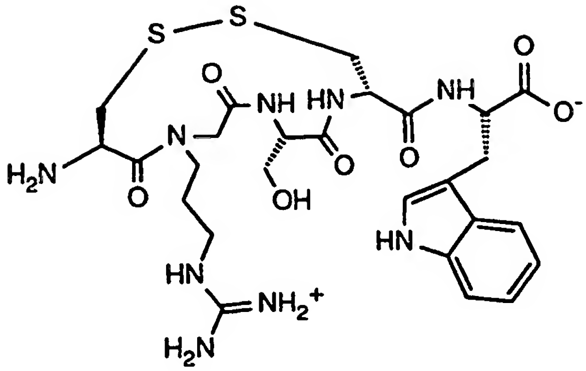
10. A heterocyclic compounds of the formula (3), (4), (5), (6), (7), (8) or (9)



(3)



(4)



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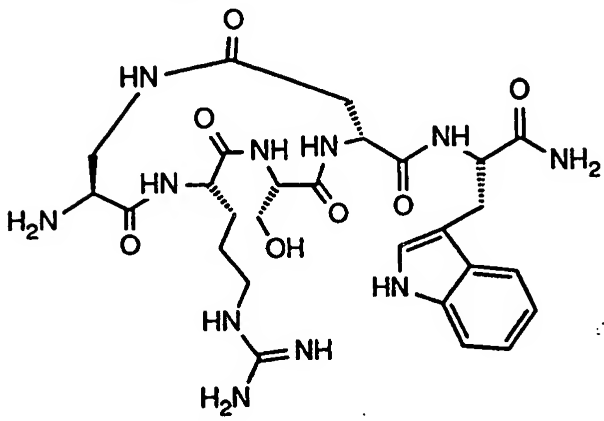

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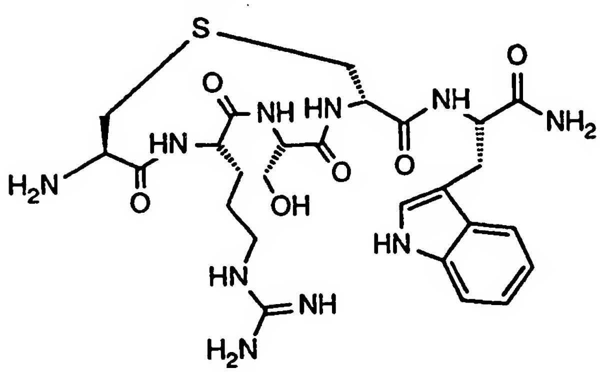

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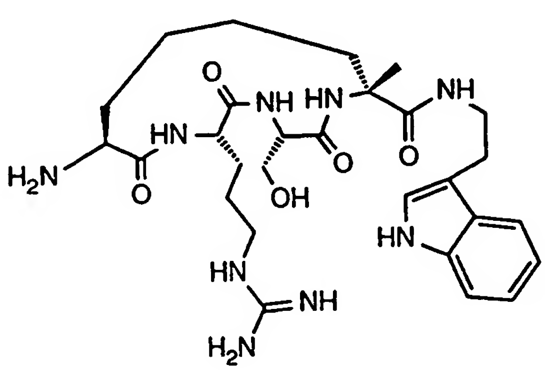

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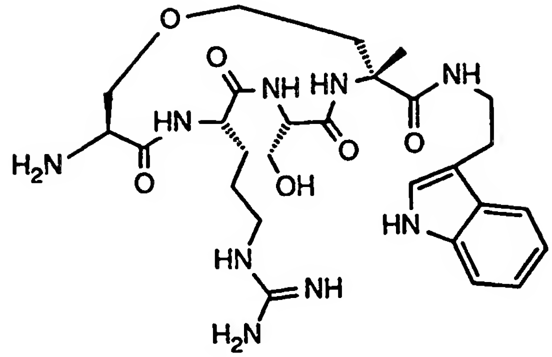
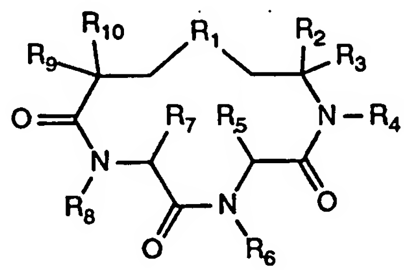


(9)

11. A heterocyclic compound according to claim 1 of the formula (2)



(2)

wherein

$R_1 = CH_2, C_2H_4, C_2H_2, CH(CH_3)\text{-}CH_2, S, CH_2\text{-}S, CH_2\text{-}S\text{-}S, S\text{-}S\text{-}CH_2, S\text{-}S, S{=}O,$ $O{=}S{=}O$, NH, NH-CO, O, Se; CH_2, C_2H_4, C_2H_2, $CH(CH_3)\text{-}CH_2$, S, CH_2-S, CH_2-S-S, S-S, S=O, O=S=O, NH, NH-CO, O, O-CH_2, CH_2-O, or Se;

$R_2 = NH_2$; C_1-C_4alkyl substituted with NH_2; —N(piperazine)NH; -CO-NH-CH_2-CO-NH_2 or -CO-NH-CH_2-CH_2-NH_2 unsubstituted or substituted with methyl or ethyl;

R_3, R_4, R_8 and R_9 independently of each other are hydrogen or C_1-C_4alkyl;

one of the residues R_5 and R_6 is C_2-C_5alkyl substituted with -C(=NH)NH_2 or -NH-C(=NH)NH_2; and the other residue is hydrogen or C_1-C_4alkyl;

R_7 = hydroxy or C_1-C_4alkyl substituted with hydroxy;

R_{10} = -NH-C_1-C_6alkyl-aryl or -NH-CO-CH(C_1-C_3alkyl-aryl)R_{11};

R_{11} = hydrogen, NH_2, -NH-CO(C_1-C_4alkyl) or -NH-R_{12};

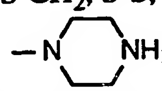
R_{12} = a radical of a naturally occurring amino acid or a radical of a peptide containing 2-5 naturally occurring amino acids.

12. A heterocyclic compound according to claim 11 wherein

R_1 = CH_2, C_2H_4, S, CH_2-S, CH_2-S-S, S-S-CH_2, S-S, NH-CO, O, O-CH_2, CH_2-O, Se;

$R_2 = NH_2$; C_1-C_4alkyl substituted with NH_2; —N(piperazine)NH; -CO-NH-CH_2-CO-NH_2;

-CO-NH-CH(CH_3)-CO-NH_2 or -CO-NH-CH_2-CH_2-NH_2;
R_3, R_4, R_8 and R_9 independently of each other are hydrogen or methyl or ethyl;
one of the residues R_5 and R_6 is C_2-C_5alkyl substituted with -C(=NH)NH_2 or -NH-C(=NH)NH_2; and the other residue is hydrogen, methyl or ethyl;
R_7 = hydroxy or C_1-C_3alkyl substituted with hydroxy;
R_{10} = -NH-C_1-C_6alkyl-R_{13} or -NH-CO-CH(C_1-C_3alkyl-R_{13})R_{11};
R_{11} = hydrogen, NH_2, -NH-$COCH_3$, -NH-$COCH_2$-CH_3 or -NH-R_{12};
R_{12} = a radical of a naturally occurring amino acid;
R_{13} = phenyl, hydroxyphenyl, naphthyl, indolyl, pyridyl, quinolyl, isoquinolyl or pyrrolyl.

13. A heterocyclic compound according to claim 11 wherein
R_1 = CH_2, CH_2-CH_2, S, CH_2-S, CH_2-S-S, S-S-CH_2, S-S, NH-CO, O, O-CH_2, CH_2-O;
R_2 = NH_2; C_1-C_4alkyl substituted with NH_2; ; -CO-NH-CH_2-CO-NH_2; -CO-NH-CH(CH_3)-CO-NH_2 or -CO-NH-CH_2-CH_2-NH_2;
R_3, R_4, R_8 and R_9 independently of each other are hydrogen, methyl or ethyl;
one of the residues R_5 and R_6 is C_2-C_5alkyl substituted with -C(=NH)NH_2 or -NH-C(=NH)NH_2; and the other residue is hydrogen, methyl or ethyl;
R_7 = hydroxy, hydroxymethyl or hydroxyethyl;
R_{10} = -NH-C_3-C_6alkyl-R_{13} or -NH-CO-CH(C_1-C_3alkyl-R_{13})R_{11};
R_{11} = hydrogen, NH_2, -NH-$COCH_3$, -NH-$COCH_2$-CH_3 or -NH-R_{12};
R_{12} = a radical of a naturally occurring amino acid;
R_{13} = phenyl, hydroxyphenyl, naphthyl, indolyl, pyridyl, quinolyl, isoquinolyl or pyrrolyl.

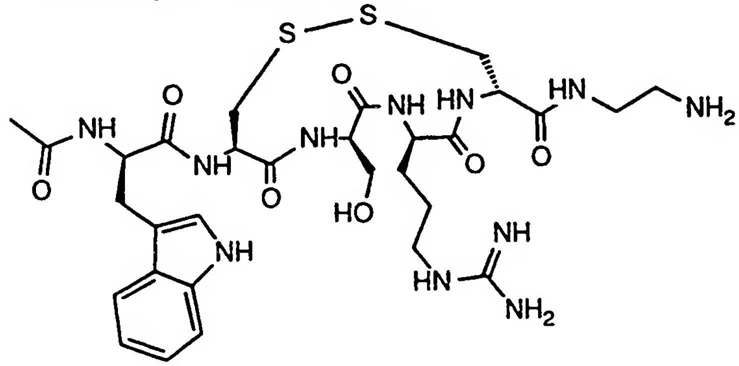
14. A heterocyclic compound according to claim 11 wherein
R_1 = CH_2, CH_2-CH_2, S, CH_2-S, CH_2-S-S, S-S-CH_2, S-S, NH-CO, O, O-CH_2, CH_2-O;
R_2 = NH_2; -CH_2-NH_2; -CH_2-CH_2-NH_2; -CO-NH-CH_2-CO-NH_2; -CO-NH-CH(CH_3)-CO-NH_2; or -CO-NH-CH_2-CH_2-NH_2;
R_3, R_4, R_8 and R_9 independently of each other are hydrogen or methyl;
one of the residues R_5 and $R_6$is hydrogen or methyl and the other is C_2-C_4alkyl substituted with -C(=NH)NH_2 or -NH-C(=NH_2)NH_2;
R_7 = hydroxy; hydroxymethyl or hydroxyethyl;
R_{10} = -NH-C_3-C_6alkyl-R_{13} or -NH-CO-CH(C_1-C_3alkyl-R_{13})R_{11};
R_{11} = hydrogen, NH_2, -NH-$COCH_3$ or -NH-R_{12};

R_{12} = a radical of a naturally occurring amino acid;
R_{13} = phenyl, hydroxyphenyl or indolyl;

15. A heterocyclic compound according to claim 11 wherein
R_1 = S, CH_2-CH_2, CH_2-S, S-S, CH_2-S-S, NH-CO, O-CH_2, CH_2-O;
R_2 = NH_2, -CH_2-NH_2, -CH_2-CH_2-NH_2, -CO-NH-CH_2-CO-NH_2, -CO-NH-CH(CH_3)-CO-NH_2 or -CO-NH-CH_2-CH_2-NH_2;
R_3, R_4 and R_8 = hydrogen;
one of the residues R_5 and $R_6$is hydrogen and the other is -CH_2-CH_2-CH_2-NH-C(=NH)NH_2;
R_7 = hydroxy, hydroxymethyl or hydroxyethyl;
R_9 = hydrogen or methyl;
R_{10} = -NH-CO-CH(C_1-C_3alkyl-R_{13})R_{11};
R_{11} = hydrogen, NH_2 or -NH-$COCH_3$;
R_{13} = phenyl, hydroxyphenyl or indolyl;

16. A heterocyclic compound according to claim 11 wherein
R_1 = S, CH_2-S, CH_2-S-S, S-S, NH-CO;
R_2 = -CH_2-NH_2, -CO-NH-CH_2-CO-NH_2 or -CO-NH-CH_2-CH_2-NH_2;
R_3, R_4, R_6 and R_8 = hydrogen;
R_5 = -CH_2-CH_2-CH_2-NH-C(=NH)NH_2;
R_7 = hydroxy or hydroxymethyl;
R_9 = hydrogen or methyl;
R_{10} = -NH-CO-CH(CH_2-R_{13})R_{11};
R_{12} = NH_2 or -NH-$COCH_3$;
R_{13} = indolyl.

17. A heterocyclic compound of formula (10)



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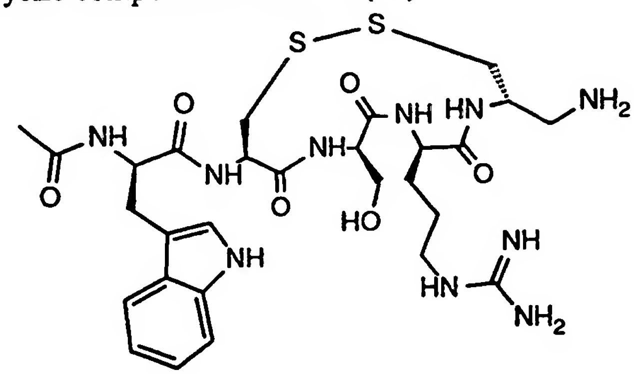
18. A heterocyclic compound of formula (11)



(11)

19. A pharmaceutical composition which consists of one or more than one of the compounds of the formula (1) as claimed in claim 1, or which comprises said compounds together with conventional auxiliaries, typically carriers and diluents.

20. A compound of formula (1) as claimed in claim 1 for use in a method for the therapeutic treatment of the human or animal body.

21. A compound of formula (1) for use in a method of treating diseases associated with the stimulation of bone resorption and restricted calcium excretion.

22. Use of a compound of the general formula (1) as claimed in claim 1 and conventional carriers for the preparation of pharmaceutical compositions.

23. Process for the production of a compound of the formula (1) according to claim 1 comprising

a) reacting an amide bond forming first fragment of a compound of formula (1) with a second amide bond forming fragment of a compound of formula (1), said first fragment and said second fragment being complementary to one another such that an amide bond is formed between said first and second fragments to result in said compound of formula (1), one of said first and second fragments containing a reactive free carboxy group and sulfoxy group, respectively, or a reactive carboxylic acid or sulfonic acid derivative thereof, and the other of said first and second fragments containing a free amino group or a reactive derivative thereof, wherein free functional groups in the mentioned fragments, with the exception of the two groups participating in the reaction, are, if necessary, in protected form,

b) cyclization of the linear precursor,

c) removing protecting groups which may be present; before or after the cyclization, and,

d) if desired, converting a salt obtainable in accordance with the process into the free compound and/or converting a free compound obtainable in accordance with the process into a salt.

A. CLASSIFICATION OF SUBJECT MA[illegible]
IPC 5 C07K5/08 C0[illegible]/10 C07K7/06 A61K37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,92 10511 (THE UNIVERSITY OF MELBOURNE) 25 June 1992 cited in the application see page 3, line 4 - page 7, line 34 see page 8, line 7 - line 17 see page 20, line 11 - line 21; claims 1-7,12-21; examples ---	4-23
A	US,A,5 149 779 (M. CHOREV AND E. ROUBINI) 22 September 1992 see the whole document ---	4-23
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 August 1994	18.08.94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fuhr, C

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF MEDICINAL CHEMISTRY vol. 28, no. 7 , July 1985 , WASHINGTON US pages 967 - 970 S.J. HOCART ET AL. 'Improved Antagonists of Luteinizing Hormone-Releasing Hormone Modified in Position 7' * compound XVI * see table III * results and discussion on pages 968-969 * -----	11

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-3
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

See annex

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Claims searched completely : 4-23

Claims not searched : 1-3

In view of the extreme large number of compounds falling under the claims 1-3, and of the absence of any sensible support for these claims in the description, the Search Division considers that it is not economically reasonable to draw a search report covering the entire subject matter of claims 1-3 (See Art. 17.2 aiii).

The search has therefor been limited to the claims 4-23, and includes all the real examples given in the description.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9210511	25-06-92	AU-A-	9063291	08-07-92
		EP-A-	0561877	29-09-93
		HU-A-	64565	28-01-94
		JP-T-	6503330	14-04-94
US-A-5149779	22-09-92	NONE		